Food Research

An Official Publication of

The Institute of Food Technologists

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Volume 17 JULY-AUGUST, 1952 Number 4

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Welcome--

to the New Editor of Food Technology and Food Research

On June 8, 1952, the Council of the Institute of Food Technologists at its meeting at Grand Rapids, Michigan, appointed Dr. Martin S. Peterson Editor of FOOD TECHNOLOGY and FOOD RESEARCH. By action of the Council in the same session, the former Editor, Z. I. Kertesz, was appointed Consulting Editor of both journals.

Dr. Peterson, who is Chief, Scientific Publications and Reports Office, Quartermaster Food and Container Institute for the Armed Forces, Chicago, will assume these additional duties on August 1, 1952. The new Editor brings to his assignment a broad background of professional experience in the editorial field extending over the past twenty years. During this period he has served as Technical Editor at the Western Regional Research Laboratory of the USDA at Albany, California; Technical Editor of the Nebraska Agricultural Experiment Station at Lincoln, Nebraska; and as a Consultant for the Quartermaster Institute. Since 1947 Dr. Peterson has been chief of the Institute's Publication Branch and its successor, the Scientific Publications and Reports Office. His responsibilities include assistance in planning the Food and Container Institute's Research and Development Program on foods and containers, supervision of publications, editing technical reports and public relations work. To food technologists he is perhaps best known for his excellent quarterly Activities Report of the Research and Development Associates.

Dr. Peterson is a graduate of Reed College, Portland, Oregon, and obtained his Ph.D. from the University of Nebraska. Prior to his affiliation with the Government he taught English at the University of Chicago, Iowa State College, and at the University of Nebraska. He is a native of Oregon, has written a number of articles on the West and is the author of "Joaquin Miller: Literary Frontiersman," published by the Stanford University Press, and co-author of "Using Better English," published by the Ronald Press.

The new Editor's extensive experiences in editing and his many years of contact with those engaged in research on foods and agricultural products and in food technology provide a sound background for his present assignment. He may be assured of the fullhearted cooperation of the officers and members of the IFT and of his Editorial Boards. We wish him many enjoyable and successful years as Editor of FOOD TECHNOLOGY and FOOD RESEARCH.

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SOME ASPECTS OF MICROBIOLOGY APPLIED TO COMMERCIAL APPLE JUICE PRODUCTION.

VI. THE SIGNIFICANCE OF CHANGES IN THE RELATIVE INCIDENCE OF SPOILAGE ORGANISMS DURING PROCESSING

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Seager, Evans and Co., Ltd., Deptford Bridge, London, England

(Manuscript received July 25, 1951)

As a result of many routine microbiological tests carried out during apple juice processing, it has been possible from the recorded data to assess the incidence of the different species of microorganisms encountered at the various stages of processing. Most of the data that has been compiled is confined almost exclusively to the relative incidence of the various species of molds that are invariably present as spoilage organisms. Accordingly, we shall discuss at some length the theoretical considerations upon which our calculations of their incidence have been based. Later in the paper the incidence of the molds relative to the yeasts and bacteria as groups will be demonstrated, for this possesses some significance in determining whether any increase is occurring in the microbial population of the juice held in bulk in the storage tanks. Direct counts are unreliable for this purpose, owing to the difficulty in obtaining representative samples of the juice for due to the construction of the tanks (Boehi pressure tanks) it is not possible to make provision for agitating their contents. The spoilage organisms referred to in this paper and the processing methods employed have been fully described in earlier papers in this series *(1, 2)*.

THEORETICAL CONSIDERATIONS

Since mold spores are not unduly prone to damage during examination and manipulation, and exhibit little tendency to undergo gross change induced by slight variations in the medium used as a culture substratum, they offer an ideal material for study.

If we select an arbitrary substratum, such as apple juice, in which the microflora are composed of a number of mixed species, then at any given time, the numbers of each individual species present will bear a relationship to each other which we will term their "relative incidence."

We will assume as an illustration, that an apple juice agar plate shows: *Penicillium expansum*, 12 colonies; *Mucor mucedo*, 2 colonies; *Aspergillus niger*, 5 colonies; and *Fusarium*, 1 colony.

Then their relative incidence on that plate will be: *P. expansum*, 60%; *M. mucedo*, 10%; *A. niger*, 25%; and *Fusarium*, 5%.

Now there are several reasons for assuming inaccuracies in the interpretation of plate counts when an indication of the relative incidence of a mixed group of microorganisms such as the molds in their natural habitat is required.

In the first place, the total number of molds present on apples is often so large that irrespective of the method of taking samples, the latter cannot be transferred to a plate of nutrient agar without making suitable dilutions in a sterile diluent. Should the ratio of the incidence of the heaviest contaminant to the least contaminant greatly exceed the number of times the sample has been diluted, a large number of plates might have to be seeded before the organism of lowest incidence appeared on any one plate. Apart from this physical limitation, the total number of mold colonies appearing on any one plate after incubation bears no direct relation to the number of mold colonies in the original sample but merely indicates the potential number of colonies that could develop from the infected material under suitable conditions. In the case of yeasts and bacteria which, unlike the molds, do not bear aerial fruiting structures carrying large numbers of spores, a plate count does bear a direct relation to the number of colonies present on the original material.

For assessment of the relative incidence of the molds we have overcome this difficulty by examining each plate qualitatively instead of counting the number of colonies actually present, and we have recorded the frequency with which each species of organism appears on the total number of plates examined.

At each stage in the production of apple juice, commencing with the fruit itself, many hundreds of samples were taken for seeding on to nutrient apple juice agar plates after suitable dilution. Apples from the silos were pulped in sterile water, diluted, and plated out on nutrient agar. Samples of juice at each stage of processing again after suitable dilution were likewise plated out.

The frequency with which each species appeared on the plates at each processing stage was then noted. This is best illustrated by referring to a hypothetical case given in Table 1 in which it is assumed that the organisms on 5 plates were examined qualitatively. Then the number of plates on which each species appeared are as follows: *R. nigricans*, 1; *Alternaria tenuis*, 3; *P. glaucum*, 2; *P. expansum*, 5; *A. niger*, 4; *M. racemosus*, 1; *A. flavipes*, 1; and *M. mucedo*, 3.

By considering the number of different species in each plate in this manner and not the total number of colonies of each species per plate, we are able to obtain a more reliable opinion of the relative incidence of each organism. In this particular hypothetical case, the percentage relative incidence will be: *R. nigricans*, 5%; *Alternaria tenuis*, 15%; *P. glaucum*, 10%; *P. expansum*, 25%; *A. niger*, 20%; *M. racemosus*, 5%; *A. flavipes*, 5%; and *M. mucedo*, 15%.

The figures given later in this paper for the relative incidence of the molds from the fruit have been compiled from examination of plate cultures from several hundred routine examinations.

CHANGES IN THE RELATIVE INCIDENCE OF MOLDS DURING PROCESSING

We have utilized our data obtained during the course of routine microbiological investigations on the factory plant to calculate the percentage incidence of the molds at four stages of processing.

Commencing with the apples in the silo, samples were taken of the juice issuing straight from the press, from juice stored in bulk in refrigerated pressure tanks for 18 months, and finally from juice pasteurized in bottle. We have found that juice pasteurized in bottle often contains up to 2 viable mold spores per ml. but fortunately their development is entirely inhibited by the presence of 0.75 volumes of carbon dioxide. The data for construction of the table showing mold incidence in pasteurized juice has been drawn from 1-ml. samples plated out without dilution from bottles supplied to the laboratory for routine examination.

TABLE 1

Hypothetical case illustrating the method of assessing relative incidence of molds on apples based upon a qualitative examination of their appearance on seeded plates

Species identified on each plate				
Plate 1	Plate 2	Plate 3	Plate 4	Plate 5
Alternaria tenuis	*Aspergillus niger*	*Penicillium expansum*	*Alternaria tenuis*	*Penicillium expansum*
Penicillium glaucum	*Mucor racemosus*		*Penicillium expansum*	*Mucor mucedo*
Penicillium expansum	*Aspergillus flavipes*		*Mucor mucedo*	*Alternaria tenuis*
Aspergillus niger	*Mucor mucedo*		*Rhizopus nigricans*	
	Penicillium expansum		*Aspergillus niger*	
	Penicillium glaucum			

In Table 2, the incidence of individual species and also of species grouped together under genera is given. These data were compiled during the course of one processing season, but that on tank-stored juice was compiled over a period of 10 to 18 months after filling the tanks. Obviously, it would not be possible to continue such detailed observations over a period of years and indeed it is not necessary. The data that we have compiled possesses a special value and interest in that they indicate those organisms which are the most persistent during processing and the most insidious from the point of view of spoilage. Under normal circumstances, these are the only species which must be constantly maintained under observation for any signs of proliferation. This will be discussed more fully later. In the meantime, we shall proceed to discuss the method of using Table 2 and the information that may be derived therefrom.

Under Columns 1, 4, 7, and 10 (Group Incidence) are given the percentage incidence of each species within the group present at each stage of processing. For example, under Column 1 we consider only the *Penicillia* group. We find that on all the fruit examined on which these organisms were present, 58% of the *Penicillia* were *Penicillium expansum* (Link) Thom, 8% *Penicillium cyclopium* Westling, 2% *Penicillium crustosum,* Thom, etc. Now if we compare *P. expansum* with *Mucor mucedo* Linne, we shall find (Column 2) that of all the molds present on the fruit, the incidence of *P. expansum* was 35.38% compared with 1.1% for *M. mucedo.* This is termed the percentage of total incidence and represents the incidence of a particular mold when compared with all other mold isolates. Columns 3, 6, 9, and 12 compare the relative incidence of each group of molds. If, for example we wish to ascertain the percentage incidence of all the *Mucorales* as compared with all the *Penicillia* in the freshly expressed juice, then we find (Column 6) that 6.1% were *Mucorales* and 60% were *Penicillia.*

Thus we are able to show: 1, percentage incidence within the genus; 2, percentage incidence of all the species; and 3, percentage incidence of each genus.

TABLE 2

Relative incidence of mold spoilage organisms (expressed on percentage basis) in apple juice at different stages of processing

Organism	Apples in silo			Juice fresh from press			Juice in storage-tanks for 18 months			Pasteurized bottled juice		
	1	2	3	4	5	6	7	8	9	10	11	12
	Group incidence	Total incidence	Total group incidence	Group incidence	Total incidence	Total group incidence	Group incidence	Total incidence	Total group incidence	Group incidence	Total incidence	Total group incidence
Mucor mucedo Linne	22	1.10		29.50	1.80		0.0	0.00		0.0	0.00	
Mucor pyriformis Fischer	4	0.20		16.30	1.00		0.0	0.00		0.0	0.00	
Mucor racemosus Fresenius	20	1.00		16.30	1.00		100.0	0.98		0.0	0.00	
Mucor hiemalis Wehmer	2	0.10		3.20	0.20		0.0	0.00		0.0	0.00	
Zygorhyncus moelleri Vuillemin	1	0.05		0.56	0.04		0.0	0.00		0.0	0.00	
Rhizopus nigricans Ehrenberg	51	2.55		34.14	2.06		0.0	0.00		0.0	0.00	
			5.0			6.1			1.0			0.0
Aspergillus niger	26	5.98		30.00	7.50		24.0	10.08		28.0	11.76	
Aspergillus ustus	2	0.46		4.00	1.00		0.0	0.00		2.0	0.84	
Aspergillus sydowi Bainier and Sartory	12	2.76		10.00	2.50		3.0	1.26		8.0	3.36	
Aspergillus nidulans Eidam	19	4.37		18.00	4.50		10.0	4.20		16.0	6.72	
Aspergillus wentii	5	1.15		4.00	1.00		0.5	0.21		2.0	0.84	
Aspergillus flavipes Bainier and Sartory	3	0.69		4.00	1.00		0.0	0.00		0.5	0.21	
Aspergillus ochraceus Wilhelm	4	0.92		3.00	0.75		0.5	0.21		1.5	0.63	
Aspergillus fumigatus	27	6.21		22.00	5.50		62.0	26.04		41.0	17.22	
Aspergillus fischeri Wehmer	2	0.46		5.00	1.25		0.0	00.00		1.0	0.42	
			23.0			25.0			42.0			42.0

Penicillium expansum (Link) Thom	58	35.58		50.00	30.00		65.0	20.15		34.0	19.72	
Penicillium cyclopium Westling	8	4.88		10.00	6.00		1.0	0.31		11.0	6.38	
Penicillium crustosum Thom	2	1.22		8.00	4.80		2.0	0.62		10.0	5.80	
Penicillium viridicatum Westling	7	4.27		6.00	3.60		0.5	0.16		2.0	1.16	
Penicillium glabrum Wehmer	5	3.05		7.00	4.20		4.0	1.24		18.0	10.44	
Penicillium spinulosum Thom	4	2.44		1.00	0.60		1.0	0.31		8.0	4.64	
Penicillium cyaneum Bainier and Sartory	5	3.05		4.00	2.40		0.5	0.16		6.0	3.48	
Lanata-typica species	3	1.83		6.00	3.60		16.5	5.12		0.5	0.29	
Brevi-compacta species	2	1.22		1.00	0.60		0.5	0.16		0.5	0.29	
Scopulariopsis brevicaulis Bainier	2	1.22		2.00	1.20		0.0	0.00		0.0	0.00	
Paecilomyces varioti Bainier	4	2.44		5.00	3.00		9.0	2.79		10.0	5.80	
			61.0			60.0			31.0			58.0
Fusarium	10	1.10		8.00	0.72		0.0	0.00		0.0	0.00	
Alternaria tenuis	14	1.54		20.00	1.78		1.0	0.26		0.0	0.00	
Cladosporium herbarum	12	1.32		25.00	2.23		96.0	24.96		0.0	0.00	
Botrytis cinerea	33	3.63		30.00	2.67		2.0	0.52		0.0	0.00	
Oospora-lactis	7	0.77		2.00	0.18		1.0	0.26		0.0	0.00	
Oospora candida	3	0.33		.....	0.00		0.0	0.00		0.0	0.00	
Monilia sitophilia	21	2.31		15.00	1.34		0.0	0.00		0.0	0.00	
			11.0			8.9			26.0			0.0

The organisms listed in Table 2 are those that have been found associated both with the fruit and with the freshly expressed juice and have retained their viability in the latter. They may therefore be regarded as potential spoilage agents if conditions become favorable for their proliferation. Many other organisms encountered on the fruit, particularly the causative agents of various fruit rots and diseases such as *Gleosporium album* Ostrew and *Sclerotinia fructigena* Alderh. and Ruhl, have not been found to be spoilage organisms in the expressed juice and we have, therefore, disregarded them in this investigation.

The value of the data we have compiled may be judged from the following observations.

If we study each group separately, commencing with the *Mucorales*, we find that both on the fruit and in the fresh juice their incidence is very small compared with all other groups. During storage of the juice they disappear completely with the exception of *Mucor racemosus* Fresenius. In a previous paper *(3)* we have shown that this organism and also *Rhizopus nigricans* Ehrenberg are the only two members of the group capable of retaining their viability in the presence of 0.75 volumes of carbon dioxide. Both on the fruit and in the expressed juice *R. nigricans* possessed the highest incidence of the organisms within the group, yet its resistance was not sufficient to withstand the higher concentration of carbon dioxide in the tank-stored juice. Once the juice has been placed to storage, therefore, only *M. racemosus* within the group requires observation.

Among the Fungi Imperfecti, *Botrytis cinerea* (which is responsible for "dry eye rot") was the most prevalent on the fruit, and in the freshly expressed juice, it was closely followed by *Cladosporium herbarum*. The latter attained a group incidence of 96% in tank-stored juice and 24.96% of the total incidence of all organisms. This organism is well-known to be resistant to cold and has been found present on meat in cold storage. None of these organisms in this group or in the *Mucorales* were heat resistant, all being destroyed by pasteurization of the bottled juice *(4)*.

Aspergillus fumigatus (27%) and *Aspergillus niger* (26%) were most prevalent among the *Aspergilli*, both on the fruit and in the freshly expressed juice. The former organism attained a group incidence of 62% in the tank-stored juice and 41% in the pasteurized juice. In the latter its incidence as a percentage of all organisms was 17% and this was followed by *A. niger* (11%) and *Aspergillus nidulans* Eidam (6.7%). These three organisms were the most heat resistant of all the *Aspergilli* and therefore need careful observation in the stored juice and during bottling and pasteurization *(4)*.

The *Penicillia* possessed the highest incidence as a group both on the fruit and in the freshly expressed juice. In the storage tanks their incidence fell to 31% compared with 42% for the *Aspergilli*. They were, however, more heat resistant than the latter and in pasteurized juice their incidence rose to 58%. Within the group itself, *P. expansum* predominated at all stages of processing.

In an investigation such as this, fluctuations in the incidence of all organisms are likely to be observed during processing and will depend on the source and condition of the fruit, and also on the methods of sam-

pling and the number of samples taken. Despite this, however, the data we have compiled from many hundreds of observations provides an overall picture of the microflora throughout processing and does indicate without doubt those species constituting the greatest risk of inducing spoilage. With this knowledge, the work of the microbiologist is simplified considerably, and he need only devote his attention to arresting the development of those few species which possess marked resistance to low temperature and high carbon dioxide pressure and those which in tank-stored juice can produce spores with pronounced heat resistant properties.

Relative Incidence of Yeasts and Bacteria

Unfortunately, it was not possible to examine individually all the species of yeasts and bacteria that we have isolated from apple juice. Accordingly it has only been possible to compare the incidence of these organisms as groups relative to the incidence of the molds as a group. These figures are given in Table 3.

TABLE 3

Relative incidences of yeasts, molds and bacteria (expressed on percentage basis) in apple juice at different stages of processing

Group	Apples in silo	Juice from press	Clarified juice	Filtered juice	Juice in storage	Pasteurized bottled juice
Yeasts	66	81.5	89.89	99.82	99.719	0.0
Molds	8	0.1	0.07	0.17	0.280	100.0
Bacteria	26	18.4	10.04	10.01	0.001	0.0

It will be observed that the incidence of the yeasts relative to the molds showed a progressive increase throughout processing up to the time that the juice was filtered immediately prior to storage. Throughout the storage period the relative incidence remained constant. On the other hand, compared with the yeasts and molds the incidence of the bacteria diminished throughout processing. The increase in the yeast incidence more particularly during the early stages of processing may be attributed to their more rapid proliferation compared with the other organisms.

Significance of Changes in Relative Incidence in Tank-stored Juice

By comparison of the relative incidence of the yeasts to molds, an indirect indication of their rate of reproduction is provided. During actual processing prior to storage, there is no particular value to be gained from such a method, but when applied to juice in storage tanks, considerable significance can be attached to the results which would otherwise be difficult to assess. Representative sampling of juice from the Boehi pressure tanks offers difficulty, since it is not possible to provide mechanical agitation to mix the contents. In consequence, over a long period the yeast counts show extreme variation and it is difficult to determine whether proliferation is taking place. Measurement of the incidence of the yeasts relative to the molds will, however, indicate whether any increase in number of the former organisms has actually occurred. For if conditions of pressure and temperature are not sufficient to restrict development of the yeasts the mold population will certainly not increase since they are inhibited in the presence of very small concentrations of carbon dioxide. Over

a period of time fluctuations in relative incidences are bound to occur due to slight differences in sedimentation rate, in sampling errors, and in dilution errors when making plate counts. Yet, unless the relative incidence shows a regular increase, it may be assumed that the storage conditions were satisfactory for arresting development of the yeasts. Over a long period of observations we have found that the relative incidence remains fairly steady and that pressure and temperature in the juice are sufficient to restrict yeast growth.

SUMMARY

Accuracy in assessing the relative incidence of apple juice spoilage organisms is subject to several physical limitations of which the most important is the extent of dilution of the original suspension required to make a plate count.

Methods for estimating the relative incidence of microorganisms at each stage of juice processing have been discussed.

The relative incidence of the molds have been studied in detail. It has been shown that by use of the production methods employed on the plant where this investigation was carried out only a few species tend to predominate during the later stages of processing. In the storage tanks the highest incidence is shown by *Aspergillus niger* (10%), *Aspergillus fumigatus* (26%), *Penicillium expansum* (20%), and *Cladosporium herbarum* (25%). A table has been constructed (Table 2) for the molds showing 1, percentage incidence within the genus; 2, percentage incidence of all the species, and 3, percentage incidence of each genus.

The incidence of the yeasts relative to the molds showed a progressive increase throughout processing whereas the incidence of the bacteria diminished.

During bulk storage the mold content of the juice remains fairly constant since their development is restricted in the presence of only a small carbon dioxide tension. By carrying out regular determinations of the yeasts relative to the molds it is possible to ascertain whether any proliferation of the former has taken place. Direct counts are unreliable for this purpose owing to difficulty in securing representative samples of juice since the storage tanks are not provided with agitation gear.

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LITERATURE CITED

1. MARSHALL, C. R., AND WALKLEY, V. T. Some aspects of microbiology applied to commercial apple juice production. II. Microbiological control of processing. *Food Research*, **16**, 515 (1951).
2. MARSHALL, C. R., AND WALKLEY, V. T. Some aspects of microbiology applied to commercial apple juice production. III. Isolation and identification of apple juice spoilage organisms. *Food Research*, **17**, 123 (1952).
3. MARSHALL, C. R., AND WALKLEY, V. T. Some aspects of microbiology applied to commercial apple juice production. IV. Development characteristics and viability of spoilage organisms in apple juice. *Food Research*, **17**, 197 (1952).
4. MARSHALL, C. R., AND WALKLEY, V. T. Some aspects of microbiology applied to commercial apple juice production. V. Thermal death rates of spoilage organisms in apple juice. *Food Research*, **17**, 204 (1952).

EFFECT OF VARIATIONS IN BLANCHING ON QUALITY OF FROZEN BROCCOLI, SNAP BEANS, AND SPINACH[a]

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Blanching or scalding of vegetables prior to freezing is necessary to obtain products which may be held in freezer storage for several months without serious deterioration. However, many different recommendations as to the blanching medium, whether water or steam, the exact length of the blanching period, and the temperature to be attained during blanching, may be found. Methods of preparing satisfactory products are known, but research is needed to determine which procedures allow for the greatest retention of nutritive value and palatability.

No attempt will be made to review the literature on this subject since Dawson, Gilpin, and Reynolds *(5)* have so recently compiled the procedures which have been proposed for the home freezing of vegetables, fruits, and prepared foods. Instead, pertinent articles will be considered in connection with the results obtained in this study.

Blanching may be accomplished by placing a definite weight of vegetable in a known amount of boiling water and holding it for a specified length of time in the hot water. This method and blanching times of 5, 3, and 1 minutes for broccoli, snap beans, and spinach, respectively, are recommended in an Illinois Extension Circular.[b] The present study was undertaken to compare the effects of this procedure and these times with others on the quality of frozen vegetables. The pressure saucepan method was included because questions about the possibility of using it have been raised. Broccoli was blanched in hot water for 3 and 5 minutes and in a pressure saucepan. Snap beans were blanched for 3 minutes in hot water, for 2 minutes in boiling water, and in a pressure saucepan. Spinach was blanched for 1 and 2 minutes in hot water. Ascorbic acid retention, palatability of the cooked frozen vegetables, and residual enzyme activity were considered as indications of quality. The ascorbic acid contents of the vegetables were determined after harvesting, after blanching and cooling, after 20 hours in the freezing unit, and after intervals of freezer storage. The palatability of the frozen products cooked after freezing and after two or three intervals of freezer storage was rated. Catalase activity was measured qualitatively after blanching and cooling, and peroxidase activity after blanching and cooling, freezing, and intervals of freezer storage.

[a] This paper represents a portion of the thesis submitted by Winona Morelle Brower to the Graduate School of the University of Illinois in partial fulfillment of the requirements for the degree of Master of Science.

[b] Van Duyne, F. O. "How to Prepare Fruits and Vegetables for Freezing with Suggestions for Choosing Suitable Varieties." University of Illinois College of Agriculture Extension Service in Agriculture and Home Economics, Circular 602, 16 pp., 1948.

EXPERIMENTAL PROCEDURE

The Early Green Sprouting broccoli, Logan snap beans, and Blight Resistant Savoy spinach used in this study were grown under the supervision of the Department of Horticulture at the University of Illinois. The vegetables were harvested at suitable stages of maturity for freezing and were brought to the food research laboratory on the day they were picked. In all, 4 lots of snap beans were obtained from August 1 to September 14, of broccoli from October 5 to 26, and of spinach from October 20 to 31.

Preliminary treatment. The vegetables were thoroughly washed, inedible or substandard portions were cut off, and excess water was removed. Representative samples of the 4 lots of each vegetable were taken for moisture and ascorbic acid determinations on the raw broccoli, snap beans, and spinach. The remainder of each lot was divided into 2 or 3 portions and blanched in different ways.

Blanching. Samples of broccoli, snap beans, and spinach were weighed and tied up in single layers of cheesecloth so that they were contained in loosely fitting bags. Thus the samples corresponded to definite weights of raw untreated material and the weights selected allowed for the proper filling of the containers used. Details of the blanching procedures are given in Table 1. In some instances a procedure was repeated on each of the 4 lots of a vegetable to give enough samples for subsequent analyses.

Subsequent treatment. Immediately after blanching, the samples were lowered into a kettle of cold water into which cold water was running, cooled for 3 minutes, and then drained. Samples of broccoli and snap beans were partially dried on towels and packed in cellophane-lined pint containers which were then sealed with heat. Spinach was packaged in the same type of containers, which were inverted and the excess liquid was pressed out before heat-sealing.

All samples, with the exception of those from one day's work on spinach, were frozen in the freezing compartment of a home freezing unit. The temperature was $-17.8°C. \pm 1.7°$ and there was a fan to circulate the air. Because of a breakdown in the home freezer, one day's run of spinach samples had to be frozen in a freezer of the plate type where the temperature ranged from $-24°$ to $-30°C.$ on the plates. After approximately 20 hours in the freezing units all vegetable samples were removed and those to be analyzed later were placed in freezer storage at a temperature of $-17.8°C. \pm 2.8°$.

Cooking the frozen vegetables. The 50-g. samples of frozen vegetables were added to 40 ml. of boiling distilled water containing 0.6 g. of salt in a beaker covered with a watch glass. After boiling was resumed, broccoli was cooked for 5 minutes and spinach for 2.5 minutes. The beans which had been blanched for 2 minutes in boiling water were boiled for 3.5 minutes, while those blanched for 3 minutes in hot water or in a pressure saucepan were boiled for 7.5 minutes.

Palatability ratings. The cooked vegetables were rated for palatability by 3 to 5 research workers accustomed to the judging of vegetables. Scores were given for color, texture, flavor, and absence of off-flavor, using a 5-point scale where 5 corresponded to very good; 4, good; 3, fair; 2, poor; and 1, very poor. The numerical values for each characteristic were defined by adjectives to aid in judging and the total scores were obtained by adding the scores given to the four characteristics.

Analytical methods. Moisture determinations were made on the raw vegetables by drying weighed ground samples to constant weight in a vacuum oven maintained at temperatures ranging from $55°$ to $60°C.$

The ascorbic acid contents of the raw and processed samples were determined by a modification of the method of Loeffler and Ponting *(9)*. In the procedure as modified 75- or 100-g. portions of raw or treated vegetables were taken for analysis and 3% metaphosphoric acid was used to blend the vegetables.

Qualitative tests for catalase and peroxidase activity were made on the blanched and cooled vegetables using the procedures described by Joslyn *(8)*. Peroxidase activity was determined in the vegetable after 20 hours in the freezing unit and after 3 and 6 months of freezer storage and only in the case of snap beans after 9 months of freezer storage.

RESULTS AND DISCUSSION

Values for the ascorbic contents of vegetables after harvesting, blanching and cooling, 20 hours in the freezer unit, and intervals of freezer

TABLE 1

Blanching procedures used for vegetables

Vegetable	As prepared for blanching	Descriptive title of blanch	Blanched in water					
			Number of samples blanched at one time	Total weight of vegetables blanched at one time	Amount of boiling water used for blanching	Length of blanch in hot water	Length of blanch in boiling water	Temperature of water at end of blanching period
				g.	*quarts*	*minutes*	*minutes*	°*C.*
Broccoli	Pieces of heads were 1.0 to 1.5 inches in diameter and overall lengths of heads and stems were approximately 4 inches	Blanched 3 minutes in hot water	5	900	12	3	.....	94.4
		Blanched 5 minutes in hot water	5	900	12	5	.....	98.0
Snap beans	Pieces 1.5 and 2.0 inches in length	Blanched 3 minutes in hot water	7	1799	12	3	.....	92.1
		Blanched 2 minutes in boiling water	3.5	899.5	8	4.7	2	Boiling
Spinach	Leaves and .5 to 1.5 inches of stems	Blanched 1 minute in hot water	2	900	8	1	.....	89.8
		Blanched 2 minutes in hot water	2	900	8	2	.....	92.4

			Blanched in a pressure saucepan					
			Number of samples blanched at one time	Total weight of vegetables blanched at one time	Amount of boiling water in pressure saucepan	Time allowed for evacuation of air	Time required to reach 10-pound pressure	Time pan was in cold water to reduce pressure
				g.	*milliliters*	*minutes*	*minutes*	*minutes*
Broccoli	As above for broccoli	Blanched in pressure saucepan	2	360	118	2.5	1.6	0.45
Snap beans	As above for snap beans	Blanched in pressure saucepan	2	514	118	2.5	2.4	0.67

storage are presented in Table 2. The means and standard deviations of the means for the amounts of ascorbic acid present are given in mg. per g. as determined, while the figures for percent retention are calculated on the basis of the original raw weights and represent the total amounts of ascorbic acid retained. The mean ascorbic acid content of the fresh Early Green Sprouting broccoli was 1.14 ± 0.051 mg. per g.; of Logan snap beans, 0.17 ± 0.011; and of Blight Resistant Savoy spinach, 0.66 ± 0.080. These figures are similar to others reported in the literature and agree quite closely with the average values (118, 19, and 59 mg. of ascorbic acid per 100 g. of broccoli, snap beans, and spinach, respectively) calculated by Watt, Merrill, Orr, Wu, and Pecot *(12)* from published and unpublished data.

Broccoli blanched in the pressure saucepan retained more ascorbic acid after blanching and cooling, 20 hours in the freezing unit, and 3 and 6 months of freezer storage than broccoli blanched for 3 minutes in hot water, while the lots blanched for 5 minutes in hot water retained the smallest amounts of ascorbic acid. The mean percent retentions for the three blanching procedures—76, 66, and 58, respectively, are significantly different from each other as indicated by the calculated values for "F" and least significant differences.[e] Broccoli which had been blanched in the pressure saucepan for 3 minutes, in hot water, and for 5 minutes in hot water and then cooled and frozen, retained 73, 65, and 59%, respectively, of the original ascorbic acid content. Batchelder, Kirkpatrick, Stein, and Marron *(2)* found only 49% retained in frozen broccoli which had been blanched four minutes in boiling water while their figure of 72% for frozen broccoli blanched for 4.5 minutes in steam is comparable to the 73% retention obtained in the present study for frozen broccoli blanched in the pressure saucepan. In both series of experiments additional losses in the amounts of ascorbic acid present occurred during 6 months of freezer storage.

With snap beans the "F" values for effect of blanching procedures, storage periods, and dates of replication were significant at the 1% level. Snap beans blanched for 3 minutes in hot water retained significantly more ascorbic acid than those blanched for 2 minutes in boiling water or those blanched in the pressure saucepan. Less ascorbic acid was retained after freezing than after blanching and cooling and as the storage periods lengthened the percent retentions of ascorbic acid decreased. Snap beans harvested on the first day of the experiment retained significantly less ascorbic acid, a mean value of 66%, after all treatments than those picked 8 to 44 days later and beans from the last picking retained a significantly higher mean value, 83%, than those harvested on previous days. Snap beans blanched by three procedures and frozen retained from 71 to 85% of the original ascorbic acid content. During 9 months of freezer storage an additional loss of 10% occurred. The loss of 29% on freezing

[e] The statistical significance of the figures for percent retention was determined by analysis of variance using blanching procedures, storage periods, dates of the 4 replications, and interactions as sources of variation *(11)*. Since the interactions were not significant, they were pooled and used as error terms. Least significant differences were calculated in the instances where "F" values were significant.

TABLE 2

Ascorbic acid content of vegetables after harvesting, blanching and cooling, 20 hours in the freezing unit, and intervals of freezer storage

Vegetables and methods of blanching	Fresh		After blanching and cooling		After 20 hours in the freezing unit		After 3 months of freezer storage		After 6 months of freezer storage		After 9 months of freezer storage	
	Moisture content mean and standard deviation of the means	Ascorbic acid content mean and standard deviation of the means	Ascorbic acid content mean and standard deviation of the means	Retention of ascorbic acid	Ascorbic acid content mean and standard deviation of the means	Retention of ascorbic acid	Ascorbic acid content mean and standard deviation of the means	Retention of ascorbic acid	Ascorbic acid content mean and standard deviation of the means	Retention of ascorbic acid	Ascorbic acid content mean and standard deviation of the means	Retention of ascorbic acid
	%	*mg. per g.*	*mg. per g.*	*%*	*mg. per g.*	*%*	*mg. per g.*	*%*	*mg. per g.*	*%*	*mg. per g.*	*%*
Broccoli	89.8±0.26	1.14±0.051										
Blanched 3 minutes in hot water			0.76±0.044	70	0.72±0.030	65	0.76±0.040	68	0.68±0.019	62	.....	....
Blanched 5 minutes in hot water			0.67±0.045	61	0.65±0.036	59	0.63±0.019	57	0.59±0.019	53	.....	....
Blanched in pressure saucepan			0.94±0.057	83	0.82±0.062	73	0.85±0.047	75	0.81±0.052	71	.....	....
Snap beans	91.1±0.22	0.17±0.011										
Blanched 3 minutes in hot water			0.15±0.009	88	0.14±0.007	85	0.14±0.005	81	0.13±0.009	77	0.12±0.006	73
Blanched 2 minutes in boiling water			0.13±0.012[c]	78[c]	0.11±0.004[c]	71[c]	0.12±0.007	73	0.11±0.009	64	0.11±0.009	63
Blanched in pressure saucepan			0.13±0.018[c]	77[c]	0.12±0.007[c]	74[c]	0.12±0.009	69	0.12±0.010	70	0.11±0.011	65
Spinach	91.9±0.20	0.66±0.080										
Blanched 1 minute in hot water			0.54±0.062	59	0.54±0.064	62	0.48±0.061	53	0.45±0.068	51	.....	....
Blanched 2 minutes in hot water			0.44±0.044	50	0.43±0.042	48	0.38±0.062	44	0.36 ±0.063	41	.....	....

[c] Based on 3 instead of 4 replications.

after blanching 2 minutes in boiling water is similar to the 33% loss determined by Farrell and Fellers *(7)* for beans prepared for freezing by the same procedure and greater than the decrease of 18% found by Phillips and Fenton *(10)*. However in the latter study the water did not stop boiling and the total blanching period was 2 minutes.

In the case of spinach the "F" values for effect of blanching procedures and storage periods were significant at the 1% level. Spinach blanched for 1 minute in hot water retained 59% of the original ascorbic acid content after blanching and cooling, and 51% after 6 months of freezer storage. Corresponding figures for spinach blanched for 2 minutes in hot water were 50 and 41%, respectively. Bedford and Hard *(3)* found 36 to 79% of the original ascorbic acid content retained in frozen spinach. These authors also reported that a 1-minute blanching period was sufficient for the retention of ascorbic acid and that a longer scalding period did not decrease the subsequent loss of ascorbic acid during freezer storage. These findings were corroborated in the present study.

The mean scores for color, texture, flavor, and absence of off-flavor of the three vegetables blanched in different ways and cooked after various treatments as well as the mean total scores and the significance of the differences between them are given in Table 3. Broccoli which had been blanched for either 3 or 5 minutes in hot water was rated significantly higher than broccoli which had been blanched in the pressure saucepan. Apparently the pressure saucepan blanch as applied in this study gave less palatable products than the hot water blanchings. Consistent differences are indicated in the lower mean scores for color, flavor, and absence of off-flavor. Only after blanching and cooling were the lots of broccoli blanched 3 minutes considered more palatable than those blanched 5 minutes.

All the total mean scores for snap beans blanched for 3 minutes in hot water were slightly higher than the scores of the beans which had been blanched for 2 minutes in boiling water, although the only significant difference occurred after 6 months of freezer storage. The beans blanched in the pressure saucepan received significantly lower scores than those blanched by the other procedures and color was the characteristic most adversely affected. Similar results were obtained by Woodroof, Atkinson, Cecil, and Shelor *(13)* who reported that beans scalded under steam pressure were poor in texture, aroma, and color and that they lacked flavor.

The mean scores for spinach which had been blanched for 1 or 2 minutes in hot water do not show any consistent or significant differences in color, texture, flavor, or absence of off-flavor. The total mean scores are very similar. With the exceptions of broccoli and snap beans blanched in the pressure saucepan and snap beans blanched in hot water and boiling water and held in freezer storage for 9 months, the total scores of the cooked frozen vegetables were 16.0 or above indicating that they were considered good products.

The qualitative tests for catalase activity on the blanched and cooled broccoli, snap beans, and spinach were negative irrespective of the method of blanching employed. If catalase can be used as an "indicator" enzyme for determining the adequacy of blanching, the methods of blanching tested in this study appear to have resulted in sufficient heat inactivation of the

enzymes. The fact that no off-flavors, which could be attributed to underblanching, were noticed in the cooked frozen vegetables is supporting evidence. Diehl *(6)* stated that the method had proved reliable for snap beans. However, Arighi, Joslyn, and Marsh *(1)* and Bedford and Joslyn *(4)* found that catalase was quantitatively inactivated at a faster rate than the enzymes concerned in off-flavor production in spinach and in string beans, respectively.

Joslyn *(8)* stated that in the qualitative test for peroxidase activity, using guaiacol and hydrogen peroxide, both negative and trace reactions could be regarded as satisfactory evidence of effective blanching. Applying this criterion to the results obtained in the present study, only the broccoli which had been blanched 5 minutes in hot water had received sufficient blanching. However, these lots of broccoli retained the smallest amounts of ascorbic acid during the 6-month experimental period, and they were not more palatable than the lots blanched 3 minutes in hot water. Broccoli blanched in the pressure saucepan gave light positive or positive reactions in approximately 25% of the tests and that blanched 3 minutes in hot water gave reactions indicative of underblanching in approximately 50% of the tests.

Snap beans blanched 3 minutes in hot water or in the pressure saucepan gave positive tests for peroxidase activity after blanching and cooling, after 20 hours in the freezing unit, and after 3, 6, and 9 months of freezer storage. When snap beans were blanched two minutes in boiling water, approximately one-third of the tests were positive. Although the peroxidase test gave evidence of a more adequate blanch in this case, more ascorbic acid was retained in the beans blanched 3 minutes in hot water, and these beans were the most palatable. Although Bedford and Joslyn *(4)* concluded that peroxidase activity was a better index to quality in frozen string beans than catalase activity, they stated that apparently all the peroxidase activity did not need to be destroyed to obtain satisfactory retention of flavor. Woodroof, Atkinson, Cecil, and Shelor *(13)* scalded snap beans for various lengths of time in hot water and steam. Positive tests for peroxidase activity were obtained on the frozen snap beans which were considered best on the basis of palatability.

Spinach blanched for 1 minute in hot water gave positive reactions for residual peroxidase activity more often than that blanched 2 minutes in hot water. This difference could not be correlated with development of off-flavors nor destruction of ascorbic acid since the palatability scores of the spinach blanched 1 minute were equal to those of spinach blanched the longer time and the ascorbic acid retention was higher.

SUMMARY

Early Green Sprouting broccoli, Logan snap beans, and Blight Resistant Savoy spinach were prepared for freezing by blanching procedures which have been recommended or suggested for home use. Evaluation of the blanching methods employed was made by determining the retention of ascorbic acid after blanching and cooling, freezing, and intervals of freezer storage, and by rating the palatability of the cooked frozen vegetables. Residual catalase and peroxidase activities were also measured.

TABLE 3

Effect of different blanching procedures on the palatability of cooked frozen vegetables

Vegetables and treatments	Number of judgments	Mean scores				Mean total scores and significance of differences[a]		
		Color	Texture	Flavor	Absence of off-flavor	For blanching method 1 and method 2	For blanching method 1 and method 3	For blanching method 2 and method 3
Broccoli								
After 20 hours in the freezing unit								
1. Blanched 3 minutes in hot water	17	4.8	4.2	4.2	4.9	18.2*	18.2**	.....
2. Blanched 5 minutes in hot water	17	4.4	3.7	4.1	4.8	17.0	.....	17.0*
3. Blanched in pressure saucepan	17	3.6	4.0	3.9	4.6	.....	16.2	16.2
After 3 months of freezer storage								
1. Blanched 3 minutes in hot water	15	4.5	3.9	4.1	4.7	17.3	17.3**	.....
2. Blanched 5 minutes in hot water	15	4.5	3.8	4.3	4.7	17.3	.....	17.3**
3. Blanched in pressure saucepan	15	3.2	3.8	3.6	4.3	.....	14.9	14.9
After 6 months of freezer storage								
1. Blanched 3 minutes in hot water	16	4.6	4.0	4.1	4.4	17.1	17.1**	.....
2. Blanched 5 minutes in hot water	16	4.5	4.1	4.1	4.8	17.6	.....	17.6**
3. Blanched in pressure saucepan	16	3.6	3.7	3.4	4.3	.....	15.0	15.0
Snap beans								
After 20 hours in the freezing unit								
1. Blanched 3 minutes in hot water	16	4.2	4.0	4.1	4.9	17.3	17.3**	.....
2. Blanched 2 minutes in boiling water	16	4.5	3.8	3.8	4.9	17.0	.....	17.0**
3. Blanched in pressure saucepan	16	3.6	3.5	3.6	4.5	.....	15.2	15.2

After 3 months of freezer storage								
1. Blanched 3 minutes in hot water	15	3.7	3.8	3.9	4.8	16.2	16.2*	.....
2. Blanched 2 minutes in boiling water	15	3.8	3.9	3.7	4.7	16.0	.....	16.0*
3. Blanched in pressure saucepan	15	3.2	3.5	3.7	4.7	.....	15.1	15.1
After 6 months of freezer storage								
1. Blanched 3 minutes in hot water	14	3.9	4.6	3.9	4.7	17.1*	17.1**	.....
2. Blanched 2 minutes in boiling water	14	4.1	4.1	3.8	4.5	16.6	.....	16.6*
3. Blanched in pressure saucepan	14	3.1	4.2	3.5	4.4	.....	15.1	15.1
After 9 months of freezer storage								
1. Blanched 3 minutes in hot water	16	3.9	3.6	3.8	4.6	15.9	15.9**	.....
2. Blanched 2 minutes in boiling water	16	4.0	3.2	3.6	4.4	15.2	.....	15.2
3. Blanched in pressure saucepan	16	3.2	3.5	3.4	4.4	.....	14.5	14.5
Spinach								
After 20 hours in the freezing unit								
1. Blanched 1 minute in hot water	18	4.9	4.3	4.5	4.9	18.6	.....	.....
2. Blanched 2 minutes in hot water	18	5.0	4.3	4.4	4.8	18.6	.....	.....
After 3 months of freezer storage								
1. Blanched 1 minute in hot water	19	4.8	3.9	4.3	4.8	17.8	.....	.....
2. Blanched 2 minutes in hot water	19	4.9	3.7	4.1	4.7	17.5	.....	.....
After 6 months of freezer storage								
1. Blanched 1 minute in hot water	18	4.8	3.6	3.8	4.6	16.8	.....	.....
2. Blanched 2 minutes in hot water	18	4.8	3.4	4.1	4.7	17.0	.....	.....

[4]The significance of differences was determined by using Student's "t" for paired data. The sign * indicates significantly higher at the 5% level, ** at the 1% level.

Although the broccoli blanched in the pressure saucepan retained the largest amounts of ascorbic acid throughout the experimental period, the use of this method cannot be recommended because the palatability of these samples was significantly lower than the palatability of the samples blanched in hot water. On the basis of ascorbic acid retention and palatability, blanching the broccoli by immersion in boiling water and removal from the hot water after 3 minutes was found to be the most satisfactory procedure. There was no evidence for the necessity of a longer blanch in hot water since the samples blanched five minutes in hot water retained the lowest amounts of ascorbic acid.

Snap beans blanched for 3 minutes in hot water retained more ascorbic acid and were rated higher in palatability after freezing and freezer storage than beans blanched for 2 minutes in boiling water or in the pressure saucepan. These results do not preclude the possibility of the 2-minute blanch in boiling water being more satisfactory if the heating facilities were such that the water continued to boil when the vegetable was added.

With broccoli and snap beans the use of the pressure saucepan for blanching did not appear promising because of the deterioration in color and flavor of the vegetables thus processed. In general the use of a 4-quart pressure saucepan for this purpose was inconvenient since only small quantities could be blanched at one time.

Spinach blanched for 1 minute in hot water retained a significantly higher amount of ascorbic acid after blanching and cooling, freezing, and 3 and 6 months of freezer storage and was equal in palatability to that blanched 2 minutes in hot water.

Qualitative tests for catalase were negative in the 3 vegetables which had been blanched by different methods and then cooled. Positive tests for residual peroxidase activity in the vegetables after blanching and cooling, freezing, and intervals of freezer storage could not be correlated with reduced ascorbic acid retention or lowered palatability scores.

Acknowledgments

The authors wish to express their appreciation to Mr. Brayton L. Weaver for supplying the broccoli, snap beans, and spinach used in this study and to Mr. Horace W. Norton for his assistance in the statistical treatment of the data.

LITERATURE CITED

1. Arighi, A. L., Joslyn, M. A., and Marsh, G. L. Enzyme activity in frozen vegetables. *Ind. Eng. Chem.*, **28**, 595 (1936).
2. Batchelder, E. L., Kirkpatrick, M. E., Stein, K. E., and Marron, I. N. Effect of scalding method on quality of three home-frozen vegetables. *J. Home Econ.*, **39**, 282 (1947).
3. Bedford, C. L., and Hard, M. M. Effect of cooling method on the ascorbic acid and carotene content of spinach, peas, and snap beans preserved by freezing. *Proc. Am. Soc. Hort. Sci.*, **55**, 403 (1950).
4. Bedford, C. L., and Joslyn, M. A. Enzyme activity in frozen vegetables—string beans. *Ind. Eng. Chem.*, **31**, 751 (1939).
5. Dawson, E. H., Gilpin, G. L., and Reynolds, H. Procedures for home freezing of vegetables, fruits, and prepared foods. *U. S. Dept. Agr., Agr. Handbook No. 2* (1950).
6. Diehl, H. C. *The Refrigerating Data Book.* Vol. II. 1940, The American Society of Refrigerating Engineers, Menasha, Wisconsin.
7. Farrell, K. T., and Fellers, C. R. Vitamin content of green snap beans. Influence of freezing, canning, and dehydration on the content of thiamin, riboflavin, and ascorbic acid. *Food Research* **7**, 171 (1942).

8. JOSLYN, M. A. Enzyme activity—index of quality in frozen vegetables. *Food Inds.*, **18**, 1204 (1946).

9. LOEFFLER, H. J., AND PONTING, J. D. Ascorbic acid. Rapid determination in fresh, frozen, or dehydrated fruits and vegetables. *Ind. Eng. Chem., Anal. Ed.*, **14**, 846 (1942).

10. PHILLIPS, M. G., AND FENTON, F. Effects of home freezing and cooking on snap beans: thiamin, riboflavin, ascorbic acid. *J. Home Econ.*, **37**, 164 (1945).

11. SNEDECOR, G. W. *Statistical Methods.* 4th Ed., 1946, The Collegiate Press, Inc., Ames, Iowa 485 pp.

12. WATT, B. K., MERRILL, A. L., ORR, M. L., WU, W. T., AND PECOT, R. K. Composition of foods—raw, processed, prepared. *U. S. Dept. Agr., Agr. Handbook No. 8* (1950).

13. WOODROOF, J. G., ATKINSON, I. S., CECIL, S. R., AND SHELOR, E. Studies of methods of scalding (blanching) vegetables for freezing. *Georgia Exp. Sta. Bull.*, **248** (1946).

HYDROLYSIS OF FATS BY BACTERIA OF THE *PSEUDOMONAS* GENUS[a]

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It is well known that the deterioration of fats in foods may be caused by the action of certain bacteria. According to Jensen and Grettie *(8)* the rancidity produced by bacterial action most commonly involves two main types of chemical reactions, hydrolytic and oxidative. Flavor defects due to free fatty acids and oxidation products can be produced by single species capable of elaborating both hydrolytic and oxidative enzymes. This paper is devoted to the investigation of some of the factors involved in the hydrolysis of fats by members of the genus *Pseudomonas.*

Early studies on the action of microorganisms on fats have been reviewed by Jensen *(7)* and Lea *(9)*. The work of Hammer and his colleagues *(3, 6, 10)* has shown that lipolytic bacteria of the *Pseudomonas-Achromobacter* groups are common in dairy products and cause flavor defects in butter.

Laboratory methods for studying the bacterial metabolism of fats have not, in general, been too satisfactory for obtaining consistent, reproducible quantitative data. Accurate analytical methods need to be devised for measuring the amounts of metabolic products formed. Procedures for emulsifying fats in a stable state of ultra-fine particle size have been lacking. Practically all of the early studies have been made using emulsified fats of variable, uncontrolled particle size. It has been demonstrated by Frazer and Walsh *(4)* that the rate of hydrolysis of a fat by lipase is proportional to the surface area and inversely proportional to the radii of the globules. In a very fine emulsion the reaction curve approximates that of a substance in true solution. One improved technique to increase the reactive surface of fat substrates was used by Trussell and Weed *(11)* who studied the lipolysis of staphylococci. These workers employed a culture shaking machine which kept the fat particles from coalescing and provided fresh surface for enzyme reaction. In the present study a further improvement in methods for producing stable, highly dispersed, fat emulsion media is reported.

MATERIALS AND METHODS

Media

Production of stable, finely-dispersed stock emulsions containing fats in concentrations as high as 25% is described below. From such stock emulsions it was convenient to make suitable dilutions with water to provide the desired experimental strengths in the media. The diluted emulsion substrate was sterilized in Erlenmeyer flasks by auto-

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claving at 15 p.s.i.g. steam pressure for 15 minutes. A separately sterilized solution of supplementary nitrogenous and mineral nutrients was added aseptically to the sterile emulsion just prior to inoculation.

Preparation of emulsions. The technique for producing stable emulsions was a modification of the procedure described by Geyer, Mann, and Stare *(5)*. The emulsifying agent used was a soy phospholipid (Astec 4135)[b] obtained from the Associated Concentrates, Inc. Satisfactory emulsions were prepared from all of the animal and vegetable oils tested. The butter oil employed (Krafteen) was supplied by the Kraft Foods Co. Also tested were refined peanut, coconut, and soybean oils, as well as refined tallow and prime steam lard, all products being furnished by Armour and Co.

The details of a typical preparation are illustrated in the following example for producing 1000 ml. of an emulsion containing 20% butter oil. In a Waring blendor, 20 g. of the emulsifying agent, Astec 4135, was dispersed in 100 ml. of water. To this agitated mixture 200 g. of butter oil, prewarmed to 60°C., was slowly added in small portions, thus providing a preliminary emulsification. After transfer to a larger vessel, the preliminary emulsion from the blendor was cautiously diluted to 1000 ml. with warm water and heated, with continuous stirring, to 60°C. The final emulsification was conducted at 2500 p.s.i.g. in a dairy homogenizer (Cherry-Burrell Junior Viscolizer). Since the equipment was preheated with hot water, the emulsion temperature remained at approximately 60°C. during the homogenization. The product was continuously recycled and after 1 hour the emulsification was considered complete. Microscopic examination showed the oil globule diameters to be uniformly less than 0.5 micron. Emulsions so prepared do not break on autoclaving and the enormous oil surface exposed is readily available for bacterial attack. In general, concentrated emulsions containing approximately 20% fat were prepared in quantities of 3 to 6 liters, sterilized in 1 liter amounts, and stored under refrigeration until used. Such emulsions have remained stable for at least 3 months of storage.

Fat emulsion broths. Unless otherwise stated, lipolysis experiments were conducted using 300 ml. of emulsion broth per flask. To prepare 300 ml. of emulsion broth medium, the procedure adopted consisted of sterilizing in 500-ml. Erlenmeyer flasks 250-ml. portions of the emulsion suitably diluted from the stock emulsion. To this emulsion was added 50 ml. of a separately sterilized solution of supplemental nutrients adjusted to pH 6.8 with dilute hydrochloric acid. A preliminary fat analysis of the stock emulsion was made in order to prepare dilutions generally in the range of 4 to 6% fat for the final medium. The concentration of supplemental nutrients in the final medium, unless otherwise stated, consisted of 0.5% yeast extract and 0.1% each of primary and secondary potassium phosphates. Subsequent experiments have shown that secondary potassium phosphate in 0.2% concentration can be substituted for the mixture of primary and secondary phosphates.

Cultures

A variety of organisms were tested for their lipolytic power. In all, 25 cultures comprising members of the genera *Pseudomonas, Achromobacter, Alkaligenes, Candida,* and *Mycoderma* were given preliminary screening runs. One culture, *Pseudomonas fluorescens* (No. 52) was selected for detailed study in most of the experiments reported here. This culture, obtained from the collection of the Northern Regional Research Laboratory as NRRL-B12, was maintained in stock on agar slants (5% glycerol, 0.5% yeast extract, 1.8% agar) and inocula were prepared from actively growing subcultures in glycerol-yeast extract broth. The broth medium had the same composition as the solid medium except that the agar was omitted. Incubation was at 30°C. and the organisms were routinely subcultured daily for 3 or 4 days to obtain active transfers for lipolysis experiments. For the experimental runs the inoculum level employed was 3 ml. of an active 24-hour culture per 100 ml. of medium.

Isolation medium for lipolytic organisms. For detection and isolation of lipolytic organisms from food sources, an improved plating medium was devised. This plating medium, which is also suitable for estimating the numbers of lipolytic organisms in food samples, has been applied satisfactorily to such products as butter, pork trimmings, beef hamburger, dried peas, and beans. The medium consists of 1.5% butter oil in emulsion,

[b] The emulsifying agent, Astec 4135, was resistant to bacterial hydrolysis and did not contribute to the free acidity formed in the lipolytic cultures.

0.5% yeast extract, 0.2% dipotassium phosphate, 0.005% Nile blue sulfate, and 1.5% agar.

The lipolytic agar medium was prepared by combining two sterile solutions, Mixture A and Mixture B, whose compositions are described as follows: For 1000 ml. of medium, Mixture A contained 15 g. agar, 5 g. yeast extract, and 2 g. K_2HPO_4 which were dissolved in distilled water and made to a volume of 650 ml. The pH was adjusted to 7.2 with sodium hydroxide. Mixture B comprised 300 ml. of 5% butter oil emulsion plus 50 ml. of 0.1% Nile blue sulfate solution. The 5% oil emulsion used was derived by diluting a stock emulsion prepared as described above. After sterilization for 15 minutes at 15 p.s.i.g. steam pressure, the solutions (A and B) were cooled to 45° to 50°C., and combined under aseptic conditions. The final medium was thoroughly mixed before pouring into Petri plates.

To each plate was added 1 ml. of the sample dilution and 12 to 13 ml. of melted lipolytic agar medium, adequate care being taken to distribute the sample in the medium. Plates were incubated at 30-32°C. in a properly humidified incubator. Preliminary examination and colony count were made after 2 days of incubation, and a final count of lipolytic and non-lipolytic species was performed after 5 days of incubation.

The non-lipolytic colonies did not cause any change in the reddish hue of the slightly alkaline medium, and did not themselves turn blue. Lipolysis was characterized by a blue or blue-green color imparted to the individual colony and by a deeper blue zone developed in the medium immediately surrounding and underlying the colony. These zones gradually increased in size and became clearer and more transparent as incubation continued beyond the first few days.

Bacterial counts. The most probable number counts of bacteria in fat emulsion broth cultures were followed, using the procedure and probability tables of Buchanan and Fulmer *(2)*. Serial dilutions were made of the culture, and each dilution was inoculated into 5 replicate tubes of glycerol-yeast extract broth which were then incubated at 30°C. for 72 hours.

Analytical

Samples of the culture liquor were removed aseptically by pipette for analysis.

Free fatty acid. A 10-ml. portion of culture liquor was heated with 30 ml. of isopropanol and 4 to 5 drops of phenolphthalein indicator (1% alcoholic) in a 60°C. water bath. Rapid titration with 0.100 *N*. aqueous sodium hydroxide gave sharp end points. Results are expressed as ml. of 0.100 *N*. acid per 10 ml. of culture liquor.

Extractable oil. The concentration of oil in culture liquors was determined by the following procedure: First, the emulsion was broken by a cadmium salt treatment and the mixture of fatty acids and oils was separated from the aqueous phase by extraction with an immiscible solvent. Then, the free fatty acids, converted to sodium salts, were separated from the lipid fraction. The washed oils, after evaporation of solvent, were dried to constant weight. The details of the determination follow. A 10-ml. sample of uninoculated emulsion broth or culture liquor was pipetted to a 50-ml. centrifuge tube and, after addition of powdered cadmium chloride sufficient to saturate the sample, the mixture was heated to approximately 65°C. in a water bath. The tube was permitted to stand at room temperature for 2 to 3 minutes and was then centrifuged for 1 to 2 minutes at 1,000 r.p.m. Since the clear supernatant layer of lipids could not be drawn off directly without appreciable losses, a volume of 5 ml. of benzene was added and thoroughly mixed with the contents of the tube. Separation of the benzene and aqueous layers was hastened by centrifuging the tube at 1,000 r.p.m. for approximately 1 minute and then drawing off the solvent layer using a pipette attached to a hypodermic syringe. It was found necessary to repeat this extraction process 10 times in order to extract all of the lipids present. The extracts, pooled in a separatory funnel, were repeatedly washed with small volumes (5 to 8 ml.) of aqueous 3% sodium carbonate solution to remove the free fatty acids from the glyceride fraction. When the washings no longer evidenced cloudiness, the free fatty acids were considered removed from the oils. The residual sodium carbonate was next removed by repeatedly washing the oil extract with warm water (55-60°C.) and the solution of glycerides and organic solvent was dried with anhydrous sodium sulfate. The drying agent was removed by filtering with suction through Whatman No. 5 paper and the solution, transferred to a tared weighing dish, was evaporated on a boiling water bath. The residual oil, dried to constant weight, thus represented the number of grams of glycerides present in 10 ml. of sample.

Saponification. For anhydrous fats and oils the A. O. A. C. method *(1)* was used with a reflux period of 2 hours instead of the suggested 30-minute period. For determining the concentration of oil in an emulsion by the saponification method, the oil was first separated from the emulsion, as previously described for extractable oils, in order to remove the Astec emulsifying agent.

EXPERIMENTAL RESULTS

Effect of fat concentration on bacterial hydrolysis. Emulsion broth media containing butter oil concentrations varying in the range from 0.5% to 20% were inoculated with a representative lipolytic organism, *Ps. fluorescens*, No. 52. The media contained as supplements 0.5% yeast extract and 0.2% potassium phosphate. Incubation was at 30°C. and the flasks were shaken once daily. The rate of hydrolysis was followed by pH and titratable acidity measurements. The disappearance of fat from the emulsion cultures was indicated by the difference in extractable oil content before and after fermentation.

It will be seen from Figure 1 that the hydrolytic reaction at 30°C. is substantially complete at the end of 4 weeks. Plotted values are corrected acidities which represent the difference between the experimental flasks and

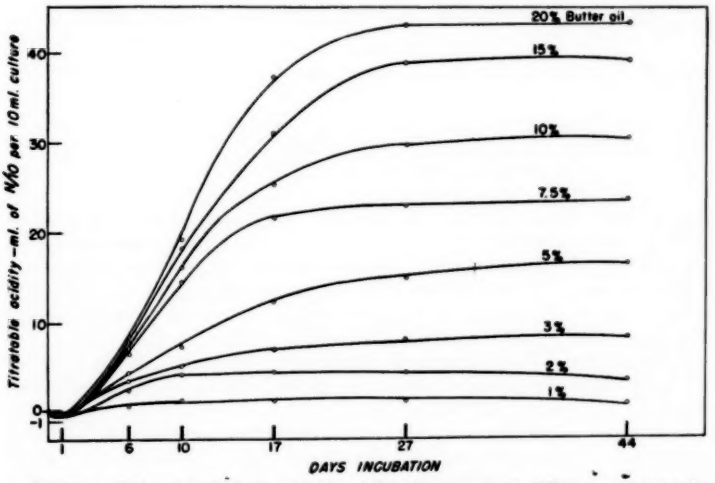

Figure 1. Rate of hydrolytic activity of Ps. fluorescens at different concentrations of butter oil.

their blanks. Control flasks are blanks consisting of uninoculated media which contained the same butter oil concentrations as these in the inoculated series. The blanks showed no significant change in acidity during the 44-day experimental period, thus indicating no hydrolysis of the oil emulsion in the absence of the lipolytic bacteria. Results are averaged from duplicate flasks. During the initial stage of incubation it has been regularly observed that a slight but definite decrease in acidity occurred before lipolysis was manifested. The dip in the acidity curve took place within the first

24 hours. Where little or no fat was present, this reduction in titratable acidity was more marked than at fat concentrations above 1%. It is presumed that the explanation for the preliminary drop in acidity is associated with the protein breakdown during the early growth of the bacterial cells. It was noted that when yeast extract, in the range varying from 0.1 to 10%, was added to emulsions with the same initial fat level, the 24-hour acidity drop increased with increasing concentrations of the nitrogenous nutrient, and the subsequent course of fermentation at protein levels above 1% yeast extract suggested that bacterial proteolysis was competing with and predominating over lipolysis.

As shown in Table 1, more than 95% of the fats were decomposed when the concentration of lipids in the emulsion media approximated the range

TABLE 1

Effect of fat concentration on hydrolysis of butter oil by Ps. fluorescens

Degree of hydrolysis		
Fat level in medium		Efficiency of hydrolysis [c]
Initial (0 days)	Final (44 days)	
g. per 100 ml.	*g. per 100 ml.*	%
20.0	7.23	63.9
10.0	1.20	88.0
7.5	0.36	95.2
5.0	0.13	97.4
1.0	0.03	96.6

[c] $\% = \frac{\text{(Initial fat concentration} - \text{Final fat concentration)}}{\text{Initial fat concentration}} \times 100$

between 1 and 8 g. per 100 ml. At higher fat levels somewhat lower lipolytic efficiencies were obtained. For the highest fat concentration tested, namely, 20 g. per 100 ml., the degree of lipolysis amounted to 63.9% of the fat originally present. The deviation from a linear relationship is apparent in Figure 2 where the degree of lipolysis, after 4 weeks of fermentation, is plotted as a function of the initial oil concentration.

Bacterial hydrolysis of various animal and vegetable fats. It has long been known that the physical properties of fats influence the degree of dispersion of oil-in-water emulsions, and that it is difficult to produce finely divided, stable emulsions with some of the higher molecular weight solid fats. If two fats of relatively different composition (butterfat and tallow, for example) can be maintained in the same state of subdivision, a comparison of the bacterial cleavage of these fats is then justified. It is suggested that the emulsification methods used by earlier investigators of bacterial lipolysis did not produce the same degree of dispersity essential for valid comparison. By neglecting these differences in dispersion, some authors concluded that the fats containing higher molecular weight fatty acids are more resistant to bacterial cleavage than those of lower molecular weight. Using emulsification procedures now available, however, the preceding concept can be re-evaluated. The experimental production of ultra-fine dispersions of fats makes possible comparisons where globule size is not a variable.

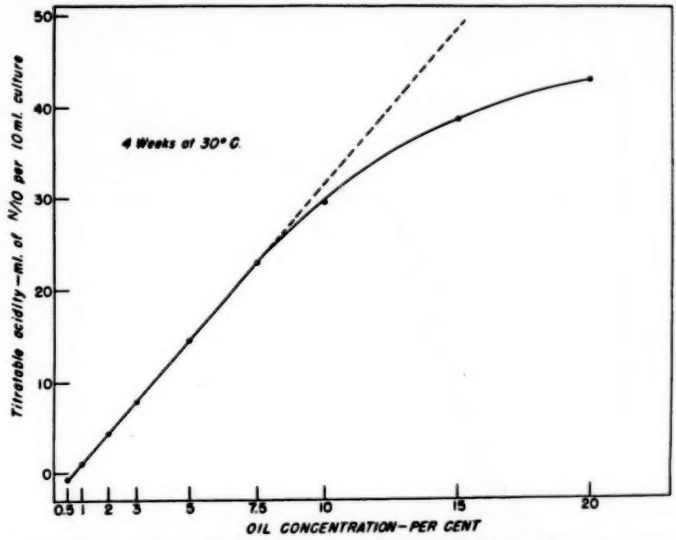

Figure 2. Effect of butter oil concentration on degree of hydrolysis by Ps. fluorescens.

In the present investigation a limited number of animal fats and vegetable oils were emulsified by the homogenizer technique described. Emulsion media containing yeast extract and potassium phosphate as supplements were seeded with active cultures of *Ps. fluorescens*, No. 52. The experimental results, shown in Table 2 and Figure 3, demonstrate that all of the fats tested were highly susceptible to microbial cleavage. Furthermore, the rate of hydrolysis followed the same general pattern for each of the different animal and vegetable fats and the high lipolytic efficiency shown for each of the oils was approximately the same.

Lipolytic activity of different Pseudomonas species. Fat-splitting enzymes have been reported in a variety of bacterial species. These include

TABLE 2

Hydrolytic action of Ps. fluorescens on different fats

Fat substrate	Saponification value	Degree of hydrolysis		
		Fat level in medium		Efficiency of hydrolysis [d]
		Initial (0 days)	Final (42 days)	
		g. per 100 ml.	*g. per 100 ml.*	%
Coconut oil	245	6.00	1.15	80.8
Butter oil	222	5.81	0.99	83.0
Tallow	189	6.05	0.96	84.1
Lard	188	7.11	1.83	74.3
Soybean oil	184	6.64	1.63	75.5
Peanut oil	183	6.25	1.31	79.0

[d] See footnote to Table 1.

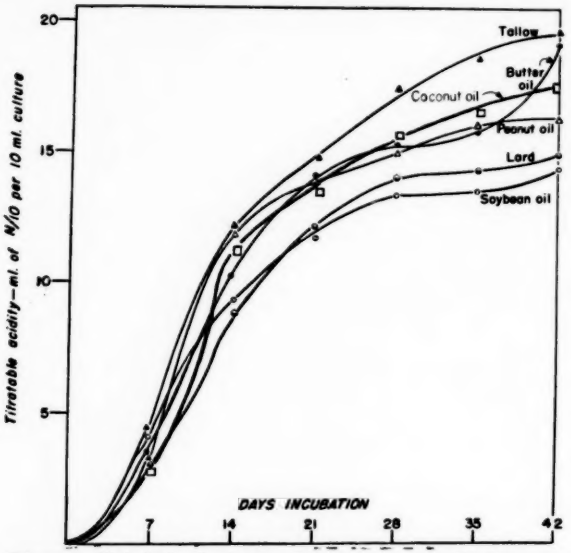

Figure 3. Hydrolysis of animal and vegetable fats by Ps. fluorescens.

members of the following genera: *Micrococcus, Bacillus, Escherichia, Corynebacterium, Pseudomonas,* and *Achromobacter (7).* In the present investigation the lipolytic activity of 8 members of the *Pseudomonas* genus was determined.

Emulsion broth media containing 5.48% butter oil, yeast extract, and potassium phosphate were inoculated with actively growing cultures of the species indicated in Table 3. At intervals during the incubation at 30°C. data were collected for titratable acidity values and pH. In addition, bacterial cell counts and extractable oil analyses were made on 4 of these cultures, as shown in Tables 3 and 4.

Wide differences in lipoclastic activity were revealed among the individual *Pseudomonas* species and between strains of the same species as shown in Table 3. Four of the cultures, Numbers 52, 57, 58, and 60, were strongly hydrolytic. Attention is called to the slow rate at which *Ps. oleovorans,* No. 60, proceeded during the first 4 weeks of incubation, and the rapid action during the next fortnight. In contrast to the dip in the acidity curve during the first day shown by *Ps. fluorescens,* there was a continuous decrease in titer for 21 days before the inflection point was reached in the case of *Ps. oleovorans.* Cultures No. 59 and No. 114 may be grouped together as organisms of only moderate lipolytic power. Based on 6-week acidity curves, Culture No. 99, *Ps. ovalis,* was classified as non-lipolytic, and Culture No. 100, *Ps. jaegeri,* was considered weakly lipolytic.

Commonly used tests for fat splitting are based on an increase in acidity after relatively short incubation periods. Slow-fermenting bacteria may be erroneously classified as non-lipolytic under the conditions of such tests.

TABLE 3

Hydrolytic activity of different Pseudomonas species on butter oil

Culture No.		Degree of hydrolysis			Rate of hydrolysis									
		Fat level in medium		Efficiency of hydrolysis [a]	Titratable acidity (corrected) [b] (Ml. of 0.1 N. per 10 ml. of Culture)									
					Days of Incubation at 30° C.									
		Initial	Final		0	2	5	7	12	14	21	28	34	42
		g. per 100 ml.	*g. per 100 ml.*	%										
52	*Ps. fluorescens*	5.48	0.51	90.7	0	0.1	0.9	7.5	11.9	11.4	15.0	16.9	17.2	18.8
57	*Ps. fragi*	5.48	1.36	75.2	0	0	1.0	2.4	5.1	6.0	9.2	11.5	13.2	15.7
99	*Ps. ovalis*	5.48	5.02	8.4	0	—0.5	—0.7	—0.8	—1.4	—1.5	—1.6	—1.7	—1.7	—1.9
100	*Ps. jaegeri*	5.48	3.74	31.7	0	—1.1	—0.4	—0.4	—0.4	—0.1	0.7	1.4	2.3	3.1
58	*Ps. fragi*	.....	.....	.....	0	0.7	0.9	2.9	5.8	6.7	12.1	14.3	15.0	16.2
59	*Ps. fragi*	.....	.....	.....	0	0.4	1.4	1.9	2.9	3.9	5.8	7.6	9.9	11.9
60	*Ps. olcovorans*	.....	.....	.....	0	—0.2	—0.7	—1.0	—1.5	—1.6	—1.8	—0.5	10.6	17.7
114	*Pseudomonas sp.* (From sweet cream)	.....	.....	.....	0	0.1	0.2	0.4	1.1	1.7	4.2	6.0	7.0	8.3

[a] See footnote to Table 1.

[b] Corrected acidity equals acidity of inoculated medium minus acidity of uninoculated medium.

TABLE 4

Bacterial growth rate in butter oil emulsion [a]

Days of Incubation	Millions of Bacteria per ml. of Culture			
	No. 52 *Ps. fluorescens*	No. 57 *Ps. fragi*	No. 99 *Ps. ovalis*	No. 100 *Ps. jaergeri*
0	350	110	25	350
2	2,500	1,300	800	3,500
5	7,000	2,500	1,100	25,000
7	1,700	2,500	1,300	18,000
12	2,500	2,500	800	5,000
14	1,300	800	500	2,500
21	35	35	350	500
28	5	13	500	800
34	0.35	0.01	130	700
42	0.001	0.00025	35	1,000

[a] Butter oil concentration, 5.48%; 30°C. incubation.

Failure to consider the incubation time factor and the occasional low acidities which fall below the initial level (negative acidity) may result in erroneous conclusions in interpreting such data. In the instance of *Ps. jaegeri*, extractable oil values showed that 31.7% of the fat had disappeared (a substantial degree of lipolysis) although acidity figures were relatively low. If a reaction time of 4 weeks were arbitrarily chosen, both *Ps. oleovorans* and *Ps. ovalis* would have fallen in the same acidity classification and have been described as non-lipolytic. At 6 weeks incubation, however, *Ps oleovorans* is grouped with the active lipolytic cultures. It has been postulated in the preceding discussion that negative acidity values result when lipolysis is masked by competing, concurrent reactions associated with proteolysis. Thus, titratable acidity figures serve as a reliable index of fat-splitting ability only when the cultures are moderately to strongly active and when the time of incubation is sufficiently prolonged.

Changes in the bacterial population of Culture Numbers 52, 57, 99, and 100 are shown in Table 4. Illustrated in Figures 4, 5, and 6 are rate of hydrolysis and bacterial growth curves. The initial lag phase of growth is not plotted, since 2 days elapsed in each case before the second count was made. In the 4 cultures studied the bacterial population rose sharply and attained a maximum in approximately 5 days. As the acidity increased, the number of viable cells declined. In the actively lipolytic cultures the pH dropped from an initial value of 6.8 to levels of 5.0 to 6.0 in 21 days, at which time the bacterial count had fallen to a point below the initial count made at the time of inoculation. At the end of 42 days a further drop in pH, reaching 4.5 to 5.0 in the strongly lipolyzing cultures, coincided with a drastic reduction in cell count. It is of interest to note that hydrolytic activity was prolonged in Cultures No. 52 and 57 despite the fact that the bacterial growth was reduced to extremely small numbers. Additional work is planned to further explore the mechanism involved.

SUMMARY

An improved procedure for studying the bacterial metabolism of fats has been developed. It includes improvement in the preparation of stable fat emulsion media and in methods of measuring bacterial cleavage. The

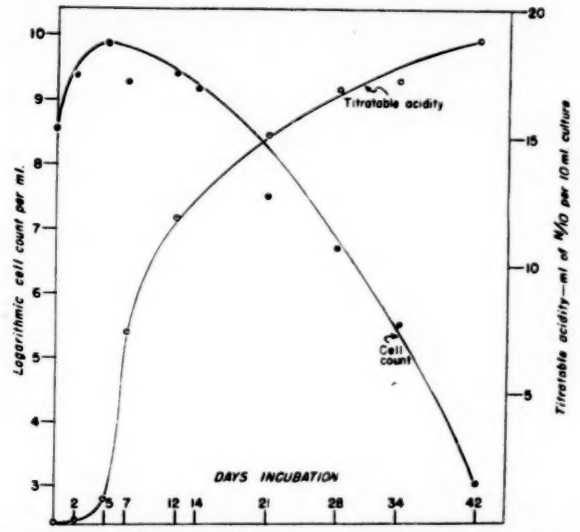
Titratable acidity
Cell count
Logarithmic cell count per ml.
Titratable acidity—ml of N/10 per 10 ml culture
DAYS INCUBATION
2 5 7 12 14 21 28 34 42

Figure 4. Rates of hydrolysis and bacterial growth in butter oil emulsion cultures of Ps. fluorescens, No. 52.

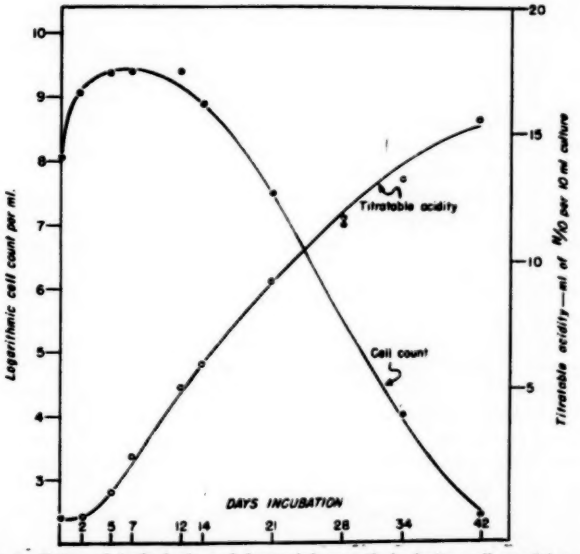
Titratable acidity
Cell count
Logarithmic cell count per ml.
Titratable acidity—ml of N/10 per 10 ml culture
DAYS INCUBATION
2 5 7 12 14 21 28 34 42

Figure 5. Rates of hydrolysis and bacterial growth in butter oil emulsion cultures of Ps. fragi, No. 57.

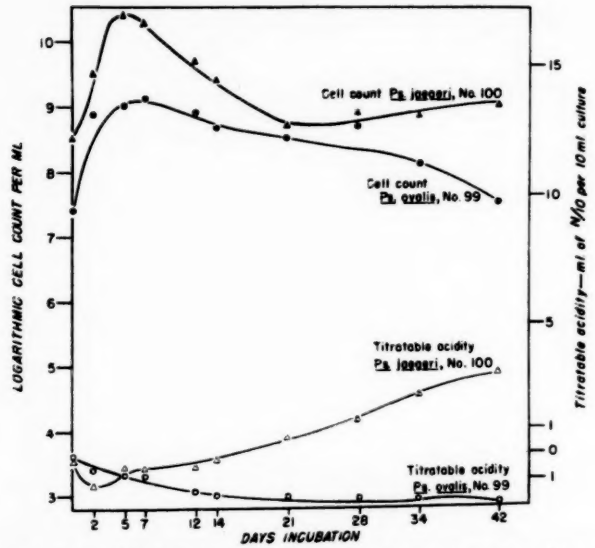
LOGARITHMIC CELL COUNT PER ML
Titratable acidity—ml of N/10 per 10 ml culture
Cell count Ps. jaegeri, No. 100
Cell count Ps. ovalis, No. 99
Titratable acidity Ps. jaegeri, No. 100
Titratable acidity Ps. ovalis, No. 99
DAYS INCUBATION

Figure 6. Rates of hydrolysis and bacterial growth in butter oil emulsion cultures of Ps. ovalis, No. 99, and Ps. jaegeri, No. 100.

importance of having finely-divided globules available in order to provide a highly effective surface for bacterial action has been demonstrated for a variety of animal and vegetable fats. Susceptibility of different fats to microbial hydrolysis appears dependent upon the particle size of the emulsified fats and not on the molecular composition of the component triglycerides. Thus, substantially the same degree of cleavage was achieved with fats possessing widely varying fatty acid compositions as butter, lard, tallow, coconut, soybean, and peanut oils.

Emulsified fats, subjected to attack by *Pseudomonas fluorescens*, undergo hydrolysis to a degree which is practically complete (90-95%) at fat concentrations below 10 g. per 100 ml. of medium. At higher fat levels up to 20 g. per 100 ml. the extent of hydrolysis deviates below the 90-95% value found in the dilute emulsions, the deviation being greater with increasing fat concentrations.

The composition of the culture medium markedly influences the type of reaction particularly for microorganisms which possess active proteolytic as well as lipolytic enzymes. When fats were supplemented by low protein levels, the *Pseudomonas* test organisms reacted mainly in a lipolytic manner. In contrast, proteolytic competition with lipolysis was indicated by lower acidity titers when larger amounts of nitrogenous supplements were added to the emulsion media.

Lipolytic powers of different strains and species of the *Pseudomonas* genus were found to vary widely in both rate and extent of hydrolysis. Of the 8 cultures tested, *Ps. fluorescens*, *Ps. olcovorans*, and 2 strains of *Ps.*

fragi were strongly lipoclastic. Another strain of *Ps. fragi* and an unidentified species of *Pseudomonas*, No. 114, isolated from cream, possessed moderate fat-splitting ability. Weak activity was shown by *Ps. jaegeri* and none was exhibited by *Ps. ovalis*.

Acknowledgment

The authors gratefully acknowledge the technical assistance of Thomas H. Burton of this Institute.

LITERATURE CITED

1. Association of Official Agricultural Chemists. *Official and Tentative Methods of Analysis*. 6th Ed., 1945, Washington, D. C.
2. Buchanan, R. E., and Fulmer, E. I. *Physiology and Biochemistry of Bacteria*. Vol. I, 1928, Williams and Wilkins Co., Baltimore, Md.
3. Collins, M. A., and Hammer, B. W. The action of certain bacteria on some simple triglycerides and natural fats, as shown by Nile-blue sulfate. *J. Bact.*, **27**, 473 (1934).
4. Frazer, A. C., and Walsh, V. G. An apparatus for the production of finely dispersed emulsions, and the rate of digestion of fat by lipase in relation to the surface area. *J. Physiol.*, **78**, 467 (1933).
5. Geyer, R. P., Manz, G. V., and Stare, F. J. Parenteral nutrition: IV. Improved techniques for the preparation of fat emulsions for intravenous nutrition. *J. Lab. Clin. Med.*, **33**, 153 (1948).
6. Hammer, B. W., and Collins, M. A. Numbers of lipolytic bacteria in various dairy products, as determined with Nile-blue sulfate. Iowa Agr. Expt. Sta., *Research Bull.*, **169**, 75 (1934).
7. Jensen, L. B. *Microbiology of Meats*. 2nd Ed., 1945, The Garrard Press, Champaign, Illinois.
8. Jensen, L. B., and Grettie, D. P. The action of microorganisms on fats. *Oil and Soap*, **10**, 23 (1933).
9. Lea, C. H. *Rancidity in Edible Fats*. 1939, Chemical Publishing Co., New York, N. Y.
10. Long, H. F., and Hammer, B. W. Methods for the detection of lipolysis by microorganisms. *Iowa State Coll. J. Sci.*, **11**, 343 (1937).
11. Trussell, R. E., and Weed, L. A. The lipolytic action of staphylococci on some pure triglycerides. *J. Bact.*, **33**, 381 (1937).

STABILITY OF ASCORBIC ACID DURING THE PREPARATION OF CRANBERRY PRODUCTS[a]

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According to Franklin *(4)* over half of the cranberry *(Vaccinium macrocarpum)* crop now goes into cans as sauce or into bottles for beverages. In 1933 Isham and Fellers *(5)* reported that fresh or boiled cold-pressed cranberry juice with or without added sugar was nearly equal to the fruit in ascorbic acid (vitamin C) content. However, when either cold-pressed or heat-extracted juice was bottled and pasteurized, most of the ascorbic acid was lost. Whole-fruit sweetened cranberry sauce retained 75-80% of the fruit's ascorbic acid. On the other hand, home and commercially made strained sauces were found to have lost practically all of the original ascorbic acid.

The purpose of this investigation was to determine at what stage in the preparation of strained cranberry sauce and cranberry cocktail the destruction of ascorbic acid occurred, and also to investigate the cause of this destruction. It was anticipated that the findings might lead to methods of improving the quality of cranberry sauce as well as having an application in the preparation of other friut products.

It is generally recognized that the presence of oxygen and metal catalysts, such as copper, are primarily responsible for the destruction of ascorbic acid during the preparation of fruit products. Cooking under vacuum and the use of an atmosphere of inert gases, such as nitrogen, have been shown to be effective methods of reducing the loss of ascorbic acid and quality in such products *(8)*. Chumarin *(2)* reported that when cranberry jam was cooked in an open kettle the ascorbic acid content dropped from 26 to 8.6 mg. % whereas vacuum cooking at 158°F.(70°C.) left 24.6 mg. % of ascorbic acid in the product. Kirk and Tressler *(6)* found that in manufacturing fruit juices, much of the ascorbic acid was lost in preparing the fruit for juicing and pressing. Heating caused an inactivation of the oxidative enzymes and also eliminated part of the dissolved air, thus reducing the extent of further oxidation. When sugar was added, the air incorporated during stirring caused a further loss of ascorbic acid.

EXPERIMENTAL

Cranberry sauce. Firm fresh cranberries were washed in cold water. They were then heated in a stainless-steel, steam-jacket kettle with an equal weight of water for 10 minutes at 200°F.(93.6°C.). The berries were occasionally stirred. The precooked berberies were next pulped by means of a Langsenkamp Juice Extractor (0.26 inch diameter screen perforation) and returned to the kettle. Hot 65% cane sugar syrup was then added in an amount which contained a weight of sugar on the dry basis equal to the

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initial weight of berries. This mixture was concentrated to a soluble solids content of 40%.

Cranberry cocktail. Frozen berries were allowed to thaw and then the juice was expressed by means of a small fruit press. The juice was clarified by passing it through an Alsop filter. Sugar was added until a 36% concentration was obtained and the resulting solution was diluted with two parts of water, filled hot into pint bottles allowing a 10% headspace, and pasteurized for 20 minutes at 160°F.(71°C.). The bottles were sealed with crown caps.

Method of sampling. Samples were taken from sauce prepared in the laboratory and also along the operational line at a commercial cranberry sauce canning plant. For sampling, 350 ml. of oxalic acid buffer solution (0.25%, pH 1.6) was placed in glass jars and the total weight of the jar and solution determined. Samples were withdrawn after each major operational step and approximately 50 g. were immediately placed in the buffer solution, the total weight was again determined, and the difference taken as the weight of the sample. This mixture was then analyzed for its ascorbic acid content. The cranberry cocktail was prepared in the laboratory and samples were taken in the same manner and analyzed for ascorbic acid. The dehydroascorbic acid content of the products was also determined in some cases.

Methods of analysis. Ascorbic acid was determined by the procedure outlined by Loeffler and Ponting *(7)*. Dehydroascorbic acid was measured by the method of Bessey *(1)* which consists essentially of reducing the dehydroascorbic acid with hydrogen sulphide gas, and measuring the increase in the indophenol reducing power.

Effect of oxygen. In order to determine the effect of preparing cranberry sauce in the absence of oxygen, a batch of sauce was prepared in a Pfaudler vacuum pan. A maximum temperature of 184°F.(84.5°C.) was attained during the process. The cooking water was previously boiled to expel any dissolved oxygen. The finishing point was ascertained by withdrawing samples and determining the soluble solids content with a refractometer.

Effect of metals. In an attempt to determine the specific effect of metals which may be present in the berries, in the various ingredients or which may be imparted by the cooking vessels or equipment, a chelating agent was added. The sauce and cocktail were prepared with the addition of Sequestrene[c] (ethylenediaminetetraacetic acid), an organic compound capable of binding metals (copper and iron) in solution, thus inactivating their catalytic power. Concentrations of 0.5% and 1.0% were employed. In the case of the sauce the Sequestrene was added directly to the cooking water, whereas in the case of the cocktail, the sequestering agent was added to the juice immediately after it was expressed.

Effect of straining. This phase was studied by the following two different procedures: 1, The precooked berries were cooled to 70°F.(21.1°C.) in a deep freeze unit operating at 0°F.(−17.7°C.) and then strained. 2, The precooked berries were strained in a stream of nitrogen gas to minimize the influence of atmospheric oxygen. Nitrogen was introduced into a large tin container by means of two rubber hoses. A dilver type strainer was set on the open end of the container and by permitting the flow of a continuous stream of nitrogen gas, the air surrounding the berries was thus largely displaced during the straining process.

Effect of different sugars. Several batches of sauce were prepared substituting dextrose, levulose, lactose, maltose, and galactose in place of sucrose to determine whether any of these sugars exerted a more or less protective action on ascorbic acid than did sucrose.

Effect of enzymes. The cranberries were ground in a galvanized food-chopper giving a fine cut and the juice was pressed out. One portion of the juice was boiled for 5 minutes to inactivate any enzymes present and the other was not treated. This latter portion served as the test sample. An equal aliquot was taken from each and placed in separate beakers. Equal amounts of a solution containing 30 mg. of ascorbic acid per 100 g. as well as some citric acid buffer of pH 3.1 were pipetted into each and the beakers were immersed in a water bath at 80°F.(27°C.). Samples were withdrawn at 15-minute intervals over a period of one hour and their ascorbic acid content was determined.

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[c]Supplied by Alrose Chemical Company, Cranston, R. I.

DISCUSSION OF THE RESULTS

The progressive loss of ascorbic acid incurred during the various operations in the manufacture of cranberry sauce is shown in Table 1. The results obtained in the laboratory show slightly higher losses than those obtained at the commercial canning plant. The loss caused by the straining process can be attributed to oxidation and this particular loss constitutes a relatively large part of the total. Likewise, the large amount of atmospheric oxygen incorporated into the sauce by the continuous stirring during the precook and finishing operations may account for a major part of the overall loss. Heat as an accelerator in the destruction must also be given some consideration especially in the last operation where a final temperature of 217°F.(102.8°C.) was attained. The slight decrease in ascorbic acid content upon the addition of sugar is attributed to the mixing process which incorporated air into the product.

When ascorbic acid (30 mg. per 100 g.) was added to heated and raw cranberry juice and juices were held at 80°F. (27°C.) for one hour, there was a 15% destruction of ascorbic acid in the raw juice while the loss in the heated juice was negligible. It would appear that the loss of ascorbic acid in the raw juice was due at least in part to the action of oxidizing enzymes. There was a progressive loss of ascorbic acid during the preparation and processing of cranberry cocktail, but little loss of ascorbic acid occurred when it was used to fortify cranberry cocktail made from heat-extracted cranberry juice (3).

As shown in Table 1, the addition of 1% Sequestrene in the preparation of strained cranberry sauce resulted in an improved retention of ascorbic acid. Although the data are not included in Table 1, the addition of 0.5 and 1.0%, respectively, of Sequestrene to raw cranberry juice as it was pressed, did not result in a higher retention of ascorbic acid in cranberry cocktail.

When the influences of oxygen and heat were minimized as by vacuum cooking, the overall retention of ascorbic acid in cranberry sauce during its production was considerably increased. Under these conditions, an 8% loss of ascorbic acid occurred in the precooking operation while an additional 8% loss occurred when the sauce was concentrated to 40% soluble solids content in a vacuum pan. This finding agrees well with those of Chumarin *(2)*.

Heat had a destructive effect on ascorbic acid in the presence of atmospheric oxygen. When the precooked berries were strained hot, 48% of the ascorbic acid was lost, but when the precooked berries were strained cold, then the loss was reduced to 16%. However, when the atmospheric oxygen was replaced with nitrogen gas, only 8% of the ascorbic acid was lost.

The influence of different sugars (sucrose, dextrose, levulose, lactose, maltose, and galactose) on the retention of ascorbic acid in the manufacture of cranberry sauce was not significant. The decrease in ascorbic acid when the various sugars were employed varied from 78-90% as compared to an 87% when sucrose was used. It is concluded that, for all practical purposes, the sugars tested exerted no better protective influence on ascorbic acid retention than did sucrose, and that in all probability the variation in results is due to a difference in the degree of aeration.

TABLE 1

Loss of ascorbic acid in various operations during the manufacture of strained cranberry sauce

Sample	Operation	Made in commercial canning plant				Made in the laboratory					
						Without Sequestrene			With 1% Sequestrene		
		Ascorbic acid content	Dehydro-ascorbic acid content	Average operational loss	Average cumulative loss	Ascorbic acid content	Average operational loss	Average cumulative loss	Ascorbic acid content	Average operational loss	Average cumulative loss
		mg. per 100 g.	*mg. per 100 g.*	%	%	*mg. per 100 g.*	%	%	*mg. per 100 g.*	%	%
1	Washing fruit	11.3	.....	.....	.....	15.7	.....	.....	11.4	.....	.....
2	Washing fruit	11.6	.....	.....	.....	15.7	.....	.....	11.4	.....	.....
3	Washing fruit	11.0	.....	.....	.....	.....	.....	.....	.....	.....	.....
1	Precooking	9.2	.....	24.0	24.0	9.5	36.5	36.5	10.0	10.0	10.0
2	Precooking	8.8	.....	.....	.....	10.3	.....	.....	10.5	.....	.....
3	Precooking	7.8	.....	.....	.....	.....	.....	.....	.....	.....	.....
1	Straining	4.4	.....	48.0	63.0	4.2	56.0	72.0	7.2	25.5	31.5
2	Straining	5.0	.....	.....	.....	4.5	.....	.....	8.2	.....	.....
3	Straining	4.0	.....	.....	.....	.....	.....	.....	.....	.....	.....
1	Addition of sugar	4.8	.....	0	59.0	3.9	3.5	72.5	7.5	+1.3	31.6
2	Addition of sugar	5.1	.....	.....	.....	4.6	.....	.....	8.1	.....	.....
3	Addition of sugar	3.8	.....	.....	.....	.....	.....	.....	.....	.....	.....
1	Concentrating to 40% soluble solids	2.0	.....	63.0	85.0	1.3	67.0	91.0	3.5	56.0	70.0
2	Concentrating to 40% soluble solids	1.4	.....	.....	.....	1.5	.....	.....	3.3	.....	.....
3	Concentrating to 40% soluble solids	1.7	.....	.....	.....	.....	.....	.....	.....	.....	.....
1	Canned sauce stored 7 days[4]	0.8	1.2	.....	93.0	.....	.....	.....	.....	.....	.....
2	Canned sauce stored 7 days	1.4	1.5	.....	.....	.....	.....	.....	.....	.....	.....
3	Canned sauce stored 7 days	0.0	3.4	.....	.....	.....	.....	.....	.....	.....	.....

[4] Stored at room temperature [70-75°F.(21.1-23.9°C.)].

SUMMARY AND CONCLUSION

Samples of cranberry sauce and cocktail were analyzed for their ascorbic acid content immediately following each unit operation in the preparation of the respective products, and the decrease was found to be progressive in both instances.

In an effort to determine the causative agent for this loss, various experiments were conducted pertaining to the relative effects induced by atmospheric oxygen, heat, metal catalysts, oxidizing enzymes, sugars, and straining the sauce. The results indicate that in the case of cranberry sauce, oxidation accelerated by atmospheric and dissolved oxygen, heat, and metal catalysts was the important factor. Enzymic oxidation may have been the destructive agent in the manufacture of the cocktail since the addition of a chelating agent did not improve ascorbic acid retention.

The addition of the chelating agent ethylenediaminetetraacetic acid (Sequestrene) resulted in a higher retention of ascorbic acid in strained cranberry sauce but not in cold-pressed cranberry cocktail.

LITERATURE CITED

1. Bessey, O. A. A method for the determination of small quantities of ascorbic acid and dehydroascorbic acid in turbid and colored solutions in the presence of sugar and other reducing substances. *J. Biol. Chem.*, **126**, 771 (1938).
2. Chumarin, N. D. Boiling jam in vacuum cookers. *Konservnaya i Plodoovoshch. Prom.*, **11** (3), 22 (1940); *C.A.*, **36**, 5277.
3. Esselen, W. B., Jr., Powers, J. J., and Fellers, C. R. The fortification of fruit juices with ascorbic acid. *Fruit Products J.*, **26**, 11 (1946).
4. Franklin, H. J. Cranberry growing in Massachusetts. Mass. Agr. Expt. Sta., *Bull.*, **447** (1948).
5. Isham, P. D., and Fellers, C. R. Effect of manufacturing and preserving processes on the vitamins of cranberries. Mass. Agr. Expt. Sta., *Bull.*, **296** (1933).
6. Kirk, M. M., and Tressler, D. K. Ascorbic acid content of pigmented fruits, vegetables, and their juices. *Food Research* **6**, 395 (1941).
7. Loeffler, H. J., and Ponting, J. D. Rapid deterioration of ascorbic acid in fresh, frozen or dehydrated fruits and vegetables. *Ind. Eng. Chem., Anal. Ed.*, **14**, 846 (1942).
8. Walker, W. S. How nitrogen protects the quality of foods. *Food Inds.*, **21**, 1189 (1949).

THE LOSS OF CHLOROPHYLL IN GREEN PEAS DURING FROZEN STORAGE AND ANALYSIS[a]

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The problem of maintaining the natural color of green vegetable foods has long occupied the attention of numerous workers, especially since the advent of the frozen food industry. Color changes which occur in frozen plant material may be caused either enzymatically or non-enzymatically. It is widely recognized that enzyme activity is not precluded by storing plant material at low temperatures; numerous enzymes have been shown to maintain their activity even in the frozen state. While it is true that the rate of enzyme reaction is lessened as the temperature is lowered, the possibility of changes in quality of frozen vegetables due to the presence and action of enzymes must always be acknowledged along with a consideration of the changes of a non-enzymatic character.

Various workers have observed that peas which had been properly blanched by immersion in boiling water, then frozen and stored at temperatures between −5.8° and 0°F.(−21° and −18°C.) retained their characteristic bright green color for extended periods of time *(6)*. If blanched peas are stored at slightly higher temperatures, that is 20°F. (−6.7°C.), though remaining in the frozen state, they will show a gradual loss of green color and a development of a greenish-brown coloration as the storage period advances. Eastmond, Peterson, and Stumpf *(7)* conducted objective measurements of the color changes in green peas with a recording spectrophotometer and photoelectric reflectometer under various conditions of processing and storage. They observed that blanched frozen peas stored at 10°F.(−12°C.) became darker, yellower, and grayer during storage than peas stored at 0° and −9.4°F.(−18° and −23°C.).

Campbell *(5)* asserted that the factor responsible for the loss of green color in frozen Alderman peas stored at insufficiently low temperatures was essentially the slow transformation of chlorophyll into pheophytin resulting from the action of the acids of the cell sap.

It has been observed on numerous occasions that raw vegetables show deterioration in color even during frozen storage at 0°F.(−18°C.). For instance, Zscheile, Beadle, and Kraybill *(31)* showed that the carotene content of certain raw vegetables, including peas, decreases during storage at this temperature.

At the present time the value of blanching green vegetables prior to placing them in frozen storage is almost universally recognized and accepted as a means of preserving the natural green color and maintaining good quality of product. However, neither the nature of the agents which

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cause losses of chlorophyll nor the mechanism of their action has been investigated in very great detail. The present study was undertaken to determine the nature of the color changes which occur in raw green peas during frozen storage, and to determine the identity of the factors responsible for these changes. In addition, the generally accepted methods for the determination of chlorophyll in plant material were examined with particular emphasis on chlorophyll losses encountered during the preparation of samples in the Waring blendor.

METHODS

The peas used in these studies were placed in cellophane bags enclosed in waxed cardboard containers and stored at 0°F(−18°C.). The procedure for chlorophyll determination was based on the work of Mackinney *(17)*. The peas were blended with an equal weight of water in a Waring blendor for 5 minutes, and 20-g. samples of the resulting slurry were then extracted several times with acetone, filtered, and the acetone concentrations adjusted to 80% in a total volume of 250 ml. The absorption of the 80% acetone extracts at 645 and 663 mμ was measured with a Beckman Model DU spectrophotometer. The optical densities at these two wave lengths were then substituted into the equations developed by Mackinney *(17)* to give the chlorophyll content of the extracts.

Lipid matter was extracted from lyophilized peas with anhydrous ethyl ether as described earlier *(13)*. Acid numbers were determined by a micro-modification of the standard A.O.A.C. procedure *(13)*. Alphahydroxy acid dehydrogenase activity was measured manometrically in a Warburg respirometer using glycolic acid as substrate *(27)*. Lipoxidase was determined by the carotene oxidase method of Reiser and Fraps *(21)*.

RESULTS AND DISCUSSION

In a study of the color changes which may occur as a result of various conditions of processing and storage, chlorophyll was determined in blanched and unblanched Thomas Laxton peas of the 1945 crop after 5 years of storage at 0°F.(−18°C.), and compared with that of similar samples of the same variety of peas from the 1950 crop analyzed immediately after harvest.

It was reported earlier by Lee and Wagenknecht *(13)* that a loss of pigments had occurred in these same raw (unblanched) peas held at 0°F.(−18°C.) for 5 years *(13,* Table 3). In contrast, peas of the same lot which had been blanched by immersion in boiling water for 1 minute and stored under identical conditions of time and temperature showed very little if any deterioration of chlorophyll. More than 60% of the chlorophyll had disappeared from the 5-year old raw peas as compared with blanched samples of the same lot held under identical storage conditions. However, all of this pigment loss did not occur during the storage period because when the above method of analysis was employed, a loss of chlorophyll of approximately 30% was observed when raw peas of the 1950 crop analyzed immediately after harvest, either fresh or frozen, were used. Blanched peas showed no such loss. It was suspected that this loss was caused by enzymatic destruction of the pigment, and furthermore that this loss occurred as a direct result of blending the pea tissue in the Waring blendor.

An attempt to prove the enzymatic nature of this chlorophyll destruction during blending was made in the following series of experiments.

The chlorophyll content of samples of two lots of raw and blanched Alderman peas, held in frozen storage at 0°F.(−18°C.) for 1 year and 6 years, respectively, was determined. Portions of the raw peas were given a standard 1-minute blanch just prior to analysis. A mixture of equal parts of raw peas and peas blanched prior to storage was also prepared. All of these samples were then blended with equal weights of water in the Waring blendor for 5 minutes and the chlorophyll content determined by the above method. The results are shown in Table 1.

TABLE 1

Chlorophyll content of Alderman peas after various storage treatments (Analyzed May, 1951)

No.	Treatment of sample	Chlorophyll, mg. per 100 g. dry weight	
		1950 crop	1945 crop
1	Unblanched, frozen	38.3	27.8
2	Unblanched, frozen; blanched prior to analysis	54.1	48.4
3	A 1 to 1 mixture of 1 and 4	45.7	34.3
4	Blanched prior to freezing and storage	68.8	61.7

From a comparison of these values for the raw peas and those blanched prior to frozen storage it was obvious that a considerable loss of chlorophyll had occurred in the raw peas during frozen storage, a loss of the same magnitude as that noted in the Thomas Laxton peas already mentioned. The difference between the chlorophyll content of the peas blanched prior to storage and those blanched immediately before analysis was taken as a measure of the amount of chlorophyll lost during storage. The amount of chlorophyll destruction in the raw peas at the end of 1 year was roughly $\frac{2}{3}$ of that found after 5 years in samples of the same variety but not of the same year of harvest. A further destruction of chlorophyll during preparation of the samples in the blendor was demonstrated by comparing the values for the raw peas with those of the peas blanched just prior to grinding in the blendor. If the destruction of chlorophyll during blending of raw peas was truly of an enzymatic nature, the mixture of equal parts of raw and blanched peas should have shown an apparent chlorophyll content smaller than the average of the values for the components of the mixture. This proved to be the case. The mixture exhibited a loss of chlorophyll approximating that observed in raw peas alone. This was taken as evidence that the destruction of chlorophyll during blending was caused enzymatically. The enzyme in the raw peas was able to bring about the destruction of some of the chlorophyll in the blanched portion of the sample in addition to its effect on that in the raw portion.

Chlorophyll is known to be acted upon by at least three enzyme systems, alphahydroxy acid dehydrogenase, chlorophyllase, and lipoxidase. The alphahydroxy acid dehydrogenase system in green plants which oxidizes glycolic and glyoxylic acids is known to cause a decolorization of chlorophyll in the course of the oxidation of the above substrates *(11, 12, 27)*. The chlorophyll is decolorized through an oxidative reaction in which it is claimed that an organic peroxide is produced. When frozen unblanched peas are blended in a Waring blendor in air an organic peroxide is formed, and a loss of chlorophyll is noted. These changes are not observed in similar treatment of blanched peas.

The possible relationships among peroxide formation, chlorophyll loss, and the enzymatic oxidation of glycolic acid were investigated in frozen unblanched Thomas Laxton peas and in fresh spinach leaves. The alphahydroxy acid dehydrogenase system was not demonstrable in homogenates of the raw peas, but it was found in fresh spinach leaf homogenates.

Chlorophyllase is a specific esterase which gives as the reaction product either the acid chlorophyllide or its simple alkyl derivative, depending on the solvent employed. This enzyme is unusual because it can function in high concentrations of ethanol (70 to 80%) and acetone (from 40 to 70%) with rates of hydrolysis comparable to its action in aqueous medium. When chlorophyll is acted on by chlorophyllase, there is a shift in the light absorption maximum for the phytol-free derivative of some 30 to 50 A units further toward the red than the maximum for chlorophyll *(28)*. Consequently on the steep portion of the curve from 6500 to 6300 A there is a very significant change in absorption.

The absorption spectra of 80% acetone extracts of raw and freshly blanched Alderman peas obtained shortly after harvesting were measured with the Beckman spectrophotometer. The raw pea extract showed a lower chlorophyll content than the blanched, which was to be expected as a result of the Waring blendor treatment during preparation of samples. If the loss of chlorophyll had been caused by the action of chlorophyllase, a spectrum shift should have been noted in the region from 6500 to 6300 A. There was no such shift, and the absorption curve for the raw peas lay directly beneath that of the blanched peas throughout the range from 7000 to 4000 A, and was typical of the spectrum for the mixed chlorophylls in peas.

Mackinney and Weast *(16)*, working with frozen peas and string beans, were unable to find evidence of the presence of chlorophyllase either in peas or string beans, and no products of hydrolysis were observed in the chlorophyll degradation products. They were unable to obtain a positive test for chlorophyllide or pheophorbide, the logical reaction products to be expected as a result of chlorophyllase action. They did, however, obtain a positive test for pheophytin in both vegetables.

By analyzing the pigments from raw frozen peas spectrophotometrically, we have been unable to demonstrate the action of chlorophyllase in bringing about this loss in chlorophyll. The pigments were extracted from the peas with low-boiling petroleum ether and chromatographed on a Tswett column of 1 to 3 MgO-Hyflo Supercel. The various pigment bands were cut out of the extruded column and redissolved in petroleum ether, and their absorption spectra in the visible range were determined in the Beckman spectrophotometer. Characteristic absorption spectra were obtained for α- and β-carotene *(19)*, chlorophyll *a* *(30)*, and pheophytin *a* *(30)*.

The lack of spectrum shift in the range of 6500 to 6300 A in the absorption curves of the raw pea extracts, indicating the absence of degradation products, was taken as evidence of the lack of chlorophyllase activity under these conditions. Willstätter *(29)* has stated that although chlorophyllase is widely distributed in chlorophyll-containing plants, there are relatively few plants which contain much of this enzyme.

Another potential source of pigment loss during storage of frozen raw peas is the conversion of chlorophyll to pheophytin *(5)*. Pheophytin is formed from chlorophyll by the action of plant acids. During prolonged frozen storage there was an increase in the acid number of the pea lipids obtained by ether extraction of lyophilized raw peas as compared with the amount present in the blanched peas *(13)*. The crude lipid fraction which amounts to 0.5-1.0% of the fresh weight of the peas, also contains the ether-extractable plant acids. This increase in acid number is shown in Table 2.

TABLE 2

Acid number of crude lipid material extracted from frozen Thomas Laxton peas

Year harvested	Storage, years	Treatment	Acid number
1950	0	Unblanched	22.6
1950	1	Unblanched	77.6
1945	5	Unblanched	97.2
1950	0	Blanched	23.0
1950	1	Blanched	20.5
1945	5	Blanched	18.6

Prior to analysis, these samples of Thomas Laxton peas had been held in storage at 0°F.(—18°C.) for 1 year and 5 years, respectively. An analysis of raw and blanched peas of the 1950 crop, analyzed immediately after harvest, is included for purposes of comparison. The greatest increase in acid number occurred during the first year of frozen storage.

The increase in acid number during frozen storage, attributed to lipase or other hydrolytic action, could enhance pheophytin formation. Thus, indirectly, lipase, whose action has been demonstrated in frozen materials *(3, 10, 14, 22)* and at temperatures as low as —22°F.(—30°C.) *(4)*, may also be involved in chlorophyll destruction during frozen storage of raw peas.

Another enzyme whose action may lead to destruction of chlorophyll is lipoxidase. When lipoxidase acts upon unsaturated fatty acids of the proper configuration, unstable intermediate fatty acid peroxides are formed. Carotenoid pigments, vitamin A, and other unsaturated compounds among the lipids may then be oxidized by these unstable intermediate products, not by direct enzymatic action nor by the relatively stable fat peroxides *(26)*. Strain has reported that chlorophylls *a* and *b* were oxidized to colorless substances by this enzyme system *(24)*.

Lipoxidase has been detected in the seeds of various legumes. The soy bean is the best known source, but the enzyme has been reported as occurring in garden peas (green and dry) *(24)*, in Alderman peas *(21, 25)*, and in Thomas Laxton peas *(21)*. Using the carotene oxidase method of Reiser and Fraps *(21)* for lipoxidase determination, we were able to demonstrate the presence of this enzyme in our frozen raw peas.

The fact that lipoxidase can function at low temperatures was shown by Lund and Halverson *(15)* who found the lipoxidase-catalyzed oxidation of sodium linoleate to be a zero order reaction in the initial stages at all temperatures between 86° and 14°F. (30° and —10°C.)

Lipid deterioration in foods containing lipoxidase cannot be adequately prevented by storage at low temperatures so long as the reactants are permitted to come in contact through a liquid medium. The lipoxidase activity is greatly reduced only when such systems are completely solidified, and the reduction in lipoxidase activity is due to the fact that the diffusion rates become the limiting factors *(15)*.

Plant tissue is not completely frozen at 0°F.(—18°C.), and the amount of unfrozen liquid present in frozen vegetables may be quite appreciable because of capillary action within the plant cells *(18)*.

Thus, although up to the present time the lipoxidase system has not been conclusively demonstrated to play a part in the destruction of chlorophyll during the frozen storage of raw peas, its involvement is strongly suspected. It is likely that lipoxidase is the causative agent for chlorophyll destruction during the maceration of raw peas in the Waring blendor in aqueous solution. The blendor affords nearly optimum conditions for lipoxidase action; enzyme and substrate are thrown into intimate contact and adequate agitation and aeration is assured.

To ascertain the oxygen requirements of the chlorophyll-destroying system, the blending of raw Alderman peas which had been stored at 0°F.(—18°C.) for 1 year was carried out in an atmosphere of nitrogen. The blendor assembly containing a mixture of equal weights of raw peas and water was enclosed beneath a large bell jar which rested upon a piece of plate glass. The electrical cord and a piece of glass tubing were sealed into a rubber stopper in the top of the bell jar. The whole system was then evacuated first by means of a water aspirator, then with a Cenco Hyvac pump. The system was then flushed with nitrogen. The evacuation and flushing were repeated 4 times. Before the final evacuation, the peas were stirred slowly by running the blendor at very low speed, by placing a variable voltage transformer in the external circuit. The peas were then blended for 5 minutes in the atmosphere of nitrogen.

Controls of raw and blanched peas were run in which the blending was carried out in air. Chlorophyll was then determined in the usual manner. From the results shown in Table 3 it is apparent that there was an additional loss of chlorophyll in the raw peas as compared to the peas blanched just prior to analysis, and further, that this loss of chlorophyll was of the same magnitude whether the peas had been blended in air or in nitrogen. The phenomenon of chlorophyll destruction under anaerobic conditions at first glance ruled out the participation of lipoxidase in the chlorophyll-destroying reaction, for oxygen is definitely known to be essential for lipoxidase action. Another example of this apparently anomalous behavior was found in the work of

TABLE 3

Effect of blending in air or in an atmosphere of nitrogen on the amount of chlorophyll found in Alderman peas

Treatment	Blending atmosphere	Chlorophyll, mg. per 100 g. dry weight
Unblanched, frozen	Air	40.7
Unblanched, frozen	Nitrogen	40.7
Unblanched, frozen; blanched prior to analysis	Air	54.1
Blanched prior to freezing and storage	Air	75.8

Irvine and Winkler *(9)*, who in a study of pigment losses during mixing of macaroni, noted that xanthophyll destruction occurred whether the mixing was carried out in air or in nitrogen. The parallelism between the loss of xanthophyll pigments during macaroni mixing and chlorophyll loss during blending of frozen raw peas was at once indicated. The lipoxidase system is saturated with oxygen at 160 mm. oxygen pressure at 32°F.(0°C.) *(8)*. Inasmuch as the oxidation of chlorophyll by lipoxidase is an induced reaction, it is conceivable that there was an ample supply of the active intermediate present in the peas prior to evacuation and flushing with nitrogen, and that the lipoxidase system could function in chlorophyll destruction under an atmosphere of nitrogen without additional oxygen. The results shown in Table 3 may best be explained on the basis of these assumptions.

The enzymatic destruction of chlorophyll during sample preparation can be prevented by employing non-aqueous solvents such as acetone in the Waring blendor, as is shown in Table 4. For this study fresh Alderman peas were obtained from the market.

Here one set each of raw and blanched peas was blended with an equal weight of water in the blendor and the other set was blended with acetone. The final concentration of the acetone extract was adjusted to 80% in each case. Through the use of acetone for the initial blending the enzymatic destruction of chlorophyll was avoided. Thus, a word of caution is in order concerning the validity of results obtained when methods for chlorophyll determination in raw plant material are based on an initial blending of the raw tissue with water in the Waring blendor prior to ace-

TABLE 4

Effect of the kind of solvent used in the blendor on the results of chlorophyll determination in Alderman peas

Treatment	Chlorophyll, mg. per 100 g. dry weight	
	Water	Acetone
Unblanched	49.8	62.1
Blanched	61.7	63.3

tone or other solvent extraction *(1, 2)*. Other workers have previously observed alterations of biological material by the Waring blendor *(20, 23)*. These include changes from the heat generated during blending, surface denaturation of proteins, and oxidation of the blended material. Preliminary studies with other green vegetables have indicated that this type of chlorophyll destruction may be dependent upon the lipoxidase content of the individual vegetables.

Work is being continued on the identification of the chlorophyll breakdown products and the enzymes involved in chlorophyll destruction and off-flavor production in frozen raw peas.

SUMMARY

A loss of chlorophyll was noted when frozen raw peas were held for periods of 1 and 5 years at 0°F.(−18°C.). In contrast, blanched peas of the same lots stored under identical conditions showed approximately equal chlorophyll content.

The enzymes lipoxidase and lipase and the large increase in acid number were postulated as being the causative agents for chlorophyll breakdown during the frozen storage of raw peas. Pheophytin *a* was identified as one of the chlorophyll degradation products. There was no evidence of chlorophyllase action in frozen raw peas.

Using a conventional method for the determination of chlorophyll, a loss was observed when raw green peas, either fresh or frozen, were blended with water in the Waring blendor. Blanched peas showed no such loss. It is possible from the evidence at hand that this loss of chlorophyll was due to lipoxidase action. The loss of chlorophyll during blending of raw peas was prevented by blanching prior to analysis or blending the sample first with 100% acetone.

It appears that present methods for chlorophyll determination should be modified to prevent the loss that occurs during the blending of raw peas.

Acknowledgment

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LITERATURE CITED

1. Arnon, D. I. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiol.*, **24**, 1 (1949).
2. Association of Official Agricultural Chemists. *Official and Tentative Methods of Analysis*. 6th Ed., 1945, 932 pp.
3. Balls, A. K., and Linkweaver, H. Action of enzymes at low temperatures. *Food Research*, **3**, 57 (1938).
4. Balls, A. K., and Tucker, I. W. Activity of lipase at low temperatures. *Ind. Eng. Chem.*, **30**, 415 (1938).
5. Campbell, H. Undesirable color changes in frozen peas stored at insufficiently low temperatures. *Food Research*, **2**, 55 (1937).
6. Diehl, H. C., and Berry, J. A. Relation of scalding practice and storage temperature to quality retention in frozen pack peas. *Proc. Am. Soc. Hort. Sci.*, **30**, 496 (1933).
7. Eastmond, E. J., Peterson, J. E., and Stumpf, R. R. Observation of color changes in some processed and stored foods. *Food Technol.*, **5**, 121 (1951).
8. Holman, R. T. Crystalline lipoxidase. II. Lipoxidase activity. *Arch. Biochem.*, **15**, 403 (1947).
9. Irvine, G. N., and Winkler, C. A. Factors affecting the color of macaroni. II. Kinetic studies of pigment destruction during mixing. *Cereal Chem.*, **27**, 205 (1950).
10. Kastle, J. H., and Loevenhart, A. S. Concerning lipase, the fat-splitting enzyme, and the reversibility of its action. *Am. Chem. J.*, **24**, 491 (1900).
11. Kolesnikov, P. A. Catalytic action of glycolic acid on oxidation of chlorophyll in ground leaves. *Doklady Akad. Nauk S.S.S.R.*, **60**, 1353 (1948).

12. KOLESNIKOV, P. A. Participation of organic peroxides and glyoxylic acid in the oxidation of chlorophyll. *Biokhimiya*, **14**, 124 (1949).
13. LEE, F. A., AND WAGENKNECHT, A. C. On the development of off-flavor during the storage of frozen raw peas. *Food Research*, **16**, 239 (1951).
14. LINEWEAVER, H. The energy of activation of enzyme reactions, and their velocity below 0°. *J. Am. Chem. Soc.*, **61**, 403 (1939).
15. LUND, A. J., AND HALVORSON, H. O. The effects of low temperatures on enzymes and microorganisms. *Proc. 3rd Conf. on Research*, Council on Research, Am. Meat Inst., Chicago, 59 (1951).
16. MACKINNEY, G., AND WEAST, C. A. Color changes in green vegetables. Frozen-pack peas and string beans. Ind. Eng. Chem., **32**, 392 (1940).
17. MACKINNEY, G. Absorption of light by chlorophyll solutions. *J. Biol. Chem.*, **140**, 315 (1941).
18. MERGENTIME, M., AND WIEGAND, E. H. Low-temperature characteristics of a pea proteinase. *Fruit Products J.*, **26**, No. 3, 72 (1946).
19. MILLER, E. S., MACKINNEY, G., AND ZSCHEILE, F. P., JR. Absorption spectra of alpha and beta carotenes and lycopene. *Plant Physiol.*, **10**, 375 (1935).
20. NAIMARK, G. M., AND MOSHER, W. A. Alteration of biological material by the Waring blendor. *J. Franklin Inst.*, **251**, 485 (1951).
21. REISER, R., AND FRAPS, G. S. Determination of carotene oxidase in legume seeds. *J. Assoc. Offic. Agr. Chemists*, **26**, 186 (1943).
22. SIZER, I. W., AND JOSEPHSON, E. S. Kinetics as a function of temperature of lipase, trypsin, and invertase activity from −70 to 50°C. (−94 to 122°F.). *Food Research*, **7**, 201 (1942).
23. STERN, R., AND BIRD, L. H. Use of the Waring blendor in biochemical work. *Biochem. J.*, **44**, 635 (1949).
24. STRAIN, H. H. Unsaturated fat oxidase; specificity, occurrence and induced oxidations. *J. Am.* Chem. Soc., **63**, 3542 (1941).
25. SUMNER, R. J. Lipoid oxidase studies. *Ind. Eng. Chem., Anal. Ed.*, **15**, 14 (1943).
26. SUMNER, R. J. Lipid oxidase studies. III. The relation between carotene oxidation and the enzymic peroxidation of unsaturated fats. *J. Biol. Chem.*, **146**, 215 (1942).
27. TOLBERT, N. E., AND BURRIS, R. H. Light activation of the plant enzyme which oxidizes glycolic acid. *J. Biol. Chem.*, **186**, 791 (1950).
28. WEAST, C. A., AND MACKINNEY, G. Chlorophyllase. *J. Biol. Chem.*, **133**, 551 (1940).
29. WILLSTÄTTER, R. Chlorophyllase. In *"Die Fermente und ihre Wirkungen."* 5th Ed., 1929, C. Oppenheimer, Ed., Georg Thiene Verlag, Leipzig, III, p. 739.
30. ZSCHEILE, F. P., AND COMAR, C. L. Influence of preparative procedure on the purity of chlorophyll components as shown by absorption spectra. *Botan. Gaz.*, **102**, 463 (1941).
31. ZSCHEILE, F. P., BEADLE, B. W., AND KRAYBILL, H. R. Carotene content of fresh and frozen green vegetables. *Food Research*, **8**, 299 (1943).

THE DEVELOPMENT OF *PSEUDOMONAS* SPOILAGE IN SHELL EGGS. I. PENETRATION THROUGH THE SHELL

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Egg spoilage due to bacteria of the genus *Pseudomonas* is the most common type of bacterial loss in cold-storage eggs, and frequently the only type found immediately after eggs are removed from the warehouse. The incidence may vary up to 80% or more in individual cases of 30 dozen eggs and entire seasons of cold storage operations have been conducted at a loss due to the high incidence of this type of spoilage.

The most common type of *Pseudomonas* spoilage and the one referred to above is usually called "sour" or "fluorescent sour," "eggs with green whites," "green rots," or "grass eggs." "Fluorescent sour" eggs, of the type referred to here, have a characteristic pattern of development. In the earliest stage there is no readily visible change and no abnormal odor; but under ultraviolet light the albumen shows a brilliant green fluorescence. Later, the albumen becomes watery and faintly green in color and the egg acquires an odor variously described as "sour," "fruity," "fishy," "cheesy," or "cabbage-water"; the fluorescence persists, but may change to bluish-green. In still later stages, one or more of the following symptoms are found: pinkish patches may appear on the shell membrane; the albumen may become stringy; the yolk may be crusted; the yolk may break and mix with the albumen.

Pennington *(13)* described and illustrated "eggs having green whites." She noted that "the shells of these eggs are frequently cracked, stained, or dirty; many have the appearance of washed eggs," and commented that they are found in greatest numbers in the spring, when dirty and wet shells are most prevalent. Large numbers of bacteria (up to 220,000,000 per g.) were found even in aseptically opened eggs in which abnormal odors had not yet developed. The predominant organism was identified as *Pseudomonas syncyanea* (Migula), recognized to be a fluorescent-pigment producing, non-gelatin-liquefying organism which fails to grow to any extent at 37°C. This author believed, however, that most of these eggs harbor mixed infections and contain *B. coli* (determined by lactose-bile fermentation) as well as gelatin liquefiers. Pennington limited use of the term "sour" to designate a non-fluorescent type of spoilage that was characterized by a pungent odor and sometimes a turbid albumen and a weakened yolk membrane, but no other readily detectable alteration. She attributed this type of spoilage to a predominately coliform infection, but altogether similar spoilage has been observed in eggs containing non-fluorescent pseudomonads. However, the term "sour" has more recently also gained currency for the fluorescent type of spoilage. Most of the other terms mentioned here have also been used for additional, entirely different egg abnormalities. To avoid further confusion, the fluorescent type of spoilage due to *Pseudomonas* infection described here, will be designated by the term "fluorescent sour."

When candled by ordinary means, eggs in the later stages of spoilage are detected with difficulty; in the earlier stages they cannot be distinguished from normal eggs. Thus, these eggs have posed a problem to the

egg packing industry. More recently the seriousness of this problem has been alleviated somewhat by the introduction of the ultraviolet candler *(10)* which permits the "fluorescent sour" condition to be detected with a high degree of accuracy, in white eggs.

Discussions of the relations of various *Pseudomonas* species and strains to this and other types of spoilage, and of the relation of egg-cleaning methods to their incidence will appear elsewhere *(8, 9, 17, 18)*. The purpose of this paper is to describe some experiments bearing on the conditions necessary for penetration of the organisms through the shell, thus permitting their growth and development in the egg contents. Information on these points may provide a basis for explaining some puzzling features in the incidence of this type of spoilage in market eggs.

LITERATURE REVIEW

Previous studies of the mechanics of egg infection have been fragmentary and, to some extent, contradictory. Most investigators are agreed, however, and have accumulated a large body of evidence to show that infection of eggs with spoilage-producing organisms very rarely, if ever, occurs before oviposition *(2, 4, 5, 6, 11, 12, 13, 15, 19)*.

Sharp and Whitaker *(16)* and Bryant and Sharp *(1)* called attention to the germicidal action of egg white against *Pseudomonas aeruginosa*. When large inoculations were made, the viable bacteria were rapidly reduced to small numbers, especially when the albumen pH was high. In no reported instance, however, were all the added bacteria killed; survivors usually established themselves and increased to large populations.

Bryant and Sharp *(1)* expressed the belief that bacterial invasion normally occurs only through the large pores in the egg shell; the existence of soiled eggs that remain uninfected was attributed to the probable lack of large pores immediately under the dirty area. Thus, washing might cause infection by spreading contamination to the large pores.

Haines and Moran *(7)* made a study of bacterial penetration through the egg shell. They were able to force a suspension of a *Pseudomonas* culture through egg shells by applying a pressure of 60 cm. of Hg. A larger organism *(Saccharomyces ellipsoideus)* also penetrated the shell under these circumstances, but less readily. They also demonstrated that cooling eggs in contact with an aqueous suspension of *Pseudomonas* organisms is an effective way of causing penetration in intact eggs, presumably as a result of the suction produced. Eggs warmed to 36.7°C. and soaked for 1 hour in a tap-water suspension were all rotted after 3 weeks at 25°C. Suction was not entirely necessary for penetration under these experimental conditions, however, for 13% spoilage occurred when there was no temperature differential, and 7% occurred when the eggs were 8.3°C. cooler than the suspension at the time of immersion.

Stuart and McNally *(19)* demonstrated virtually immediate penetration of the egg shell when half the surface of just-laid (still warm) eggs was swabbed with a liquefied gelatin culture of *Pseudomonas aeruginosa*. When such eggs were held for 192 hours at 23-28°C., fewer had infected membranes or albumens than did eggs opened 3 hours after swabbing. This result suggests the existence of limited bactericidal activity in the membrane, as well as in the albumen. They also demonstrated bactericidal activity directly by inoculating a ground saline suspension of washed shell membrane with their test organism; 80% of the cells were killed in 4 hours.

Mallman and Davidson *(11)* also studied the penetration of *P. aeruginosa* using the technique of Stuart and McNally *(19)* "except for a few (unspecified) adaptions to improve the method of culturing to lessen external contamination." They observed no infection of the membranes or egg contents, but implied that the lack of agreement of their results with that of Stuart and McNally was due to more rapid drying of the culture on the shell surface. Even when such eggs were stored in a saturated atmosphere, however, the percentage of infected eggs was relatively low.

EXPERIMENTAL METHODS

Culture used. A single strain of a *Pseudomonas* species, isolated from a typical "fluorescent sour" egg *(18)*, was used throughout the studies reported here.

Standard infection technique. For the most part, these studies were based on a method of artificial infection first described by Haines and Moran *(7)*, modified somewhat to form a "standard infection technique." Fresh, sound, "nest-clean," white eggs from a single producer were warmed to 36°C. in an incubator. Meanwhile, a 24-hour culture of the *Pseudomonas* strain in 1% yeast extract broth (Difco) was centrifuged, the supernatent broth discarded, and the bacterial cells taken up in sufficient sterile distilled water (previously chilled to 15°C.) to make a suspension containing approximately 10^6 cells per ml. The actual number of viable cells was determined by plate counts. The pre-warmed eggs were immediately and completely immersed in this suspension and allowed to remain for exactly 5 minutes, after which they were allowed to dry in the air at 20-25°C., and then stored at 15°C. The 15°C. storage temperature was chosen for all the experiments described here because it permits the most rapid development of spoilage due to infection with the experimental organism. Within the experimental storage periods employed, more fluorescent sour eggs may be detected if they have been stored at 15°C. than if stored at either higher or lower temperatures. Detailed experiments on the influence of storage temperature will be reported elsewhere.

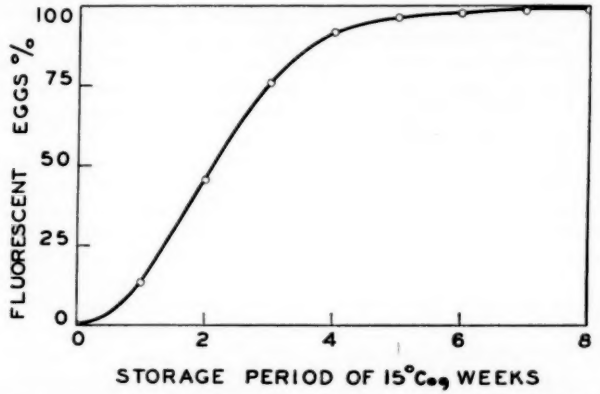

Figure 1. Development of fluorescent albumen in eggs after subjection to the standard infection technique. Points represent the average percentage of fluorescent eggs in a large number of independent experiments as determined by candling at weekly intervals with ultraviolet light. All eggs in this and subsequent figures were held at 15°C.(59°F.).

In a large number of independent experiments, nearly all eggs so treated spoiled. When candled with the ultraviolet lamp *(10)* the first fluorescent eggs may be seen in less than a week; by 2 weeks nearly 50% are spoiled and by 4 weeks over 90% (Figure 1). Variations observed in the rate and extent of spoilage development in response to variations imposed on the "standard infection technique" are some of the experimental results described below.

Detection of spoilage. During the earlier stages of this investigation the ultraviolet candler was not available, and eggs were broken at intervals to determine spoilage. Spoilage was determined either by the presence of fluorescence in the albumen of the opened eggs or by isolation of fluorescent-pigment producing organisms from the egg contents, or both. These two criteria were almost identical, because very few eggs yielded organisms that did not also have fluorescent albumen.

Since its development, the ultraviolet candler has been used routinely in these studies. By recandling the same eggs at intervals, larger sample sizes were made possible and

sampling errors were reduced. These advantages outweigh the slight loss of precision due to failure to report infected eggs that have not yet produced fluorescent albumen. These eggs are probably nearly always positive when next candled. The ultraviolet candler shows up albumen fluorescence in white shelled eggs as soon as it can be observed in an opened egg *(10)*.

Checked eggs found prior to appearance of albumen fluorescence were eliminated from consideration. The difficulty of interpreting development of spoilage in eggs with checked shells made this elimination necessary. Such eggs are apparently very much more sensitive to bacterial invasion than sound eggs, and the statistical possibility exists that bacterial invasion through cracks occurs even with dry shells.

RESULTS OF EXPERIMENTS

Effect of numbers of organisms in the infecting medium. Two experiments were performed to study the effect of concentration of bacteria. A broth culture of the type organism was taken up in distilled water in the manner already described, except that the suspension contained 1.2×10^8 viable cells per ml. This suspension was diluted serially 4 times at 1 to 100. Groups of 5 dozen eggs were treated with each of the dilutions.

Development of fluorescent albumen as determined by ultraviolet candling during the 8 weeks' storage period at 15°C. is recorded graphically in Figure 2. The number of eggs showing a fluorescent albumen at each of the successive weeks in each of the groups follow fairly smooth sigmoid curves. The rate of development and the total incidence of spoilage at the end of 8 weeks are both closely related to the concentration of organisms in the suspension up to 10^6 cells per ml.

An accurate estimate of the concentration of organisms was not made in the other experiment; but from the known growth characteristics of the organism and the dilution techniques used, the suspensions probably contained approximately the same numbers of cells as were in those used in the experiment just described. At the time of this test the penetration technique was less well standardized and spoilage was estimated by sampling (24 eggs per period). The results were thus more erratic; however, they were generally consistent with those just reported. Thus eggs immersed in suspensions containing approximately 10^6, 10^4, 10^2 and 1 cells per ml. had infected albumens to the extent of 74, 46, 37, and 0%, respectively, when opened 4 weeks later and 71, 56, 54, and 36% at the end of 9 weeks. The most concentrated suspension studied (containing approximately 10^8 cells per ml.) yielded fewer spoiled eggs than the one containing 10^6 cells in both experiments. Whether the differences should be ascribed to sampling errors, or to some such effect as clumping or matting of the cells in the pores, cannot be determined from these experiments. Apparently, however, maximal infection with this penetration technique is obtainable with a suspension containing 10^6 cells per ml.

Temperature differential between eggs and suspension. Five lots were treated with suspensions at 15, 20, 25, 30, and 35°C. All eggs were brought to 36°C. before they were immersed; thus temperature differentials of 1 to 21°C. between egg and suspension were produced. At 1, 2, 3, and 9 weeks, 24-egg samples were broken for examination. The results (Figure 3) demonstrate clearly the effect of cooling the egg in contact with a liquid suspension of the microorganism. Between differentials of 6°C. and 21°C., the percentage of infection was directly proportional to the temperature difference. When the difference was less than 6°C. this relation no longer held.

Quite likely a differential of 6°C. or less produces too little contraction and suction to effect penetration.

These curves suggest strongly that the cooling effect of the temperature differential simply affects the probability of penetration through one or more pores. The mechanism is thought to be a pressure differential set up while the egg is cooling, literally aspirating the fluid and its organisms through the pores and into the egg contents *(7)*.

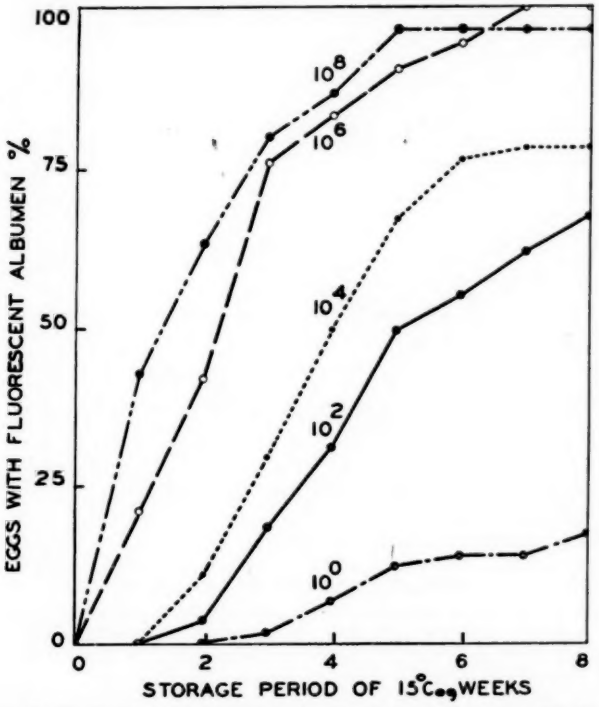

Figure 2. Development of fluorescent albumen in eggs after immersion in suspensions containing different numbers of organisms as determined by ultraviolet candling. Figures beside the curves denote the numbers of cells per ml. in the suspensions.

Penetration of organisms previously dried on the shell. In the experiment described above large numbers of organisms must have remained on the surface of the shells of all eggs. Yet if the conditions were not optimal at the time of immersion, many eggs failed to become infected. Grossly "clean" eggs are also known to carry many micro-organisms on the shell surface. The question remains whether such organisms might become sources of infection should conditions later become suitable for penetration. In a study of the behavior of organisms dried on the shell, each of three

groups of 5 dozen eggs were subjected to the following modifications of the standard infection technique: groups A and B—a suspension containing 10^6 cells per ml. was warmed to the same temperature as the eggs (36°C.); after immersion the eggs were returned to the incubator (also at 36°C.) to dry. Group A was stored at once; group B was first immersed in sterile distilled water at 15°C. for 5 minutes, and then stored. Group C was not treated with bacteria, but was immersed only in 15°C. sterile distilled water, and then stored. All eggs were stored at 15°C.

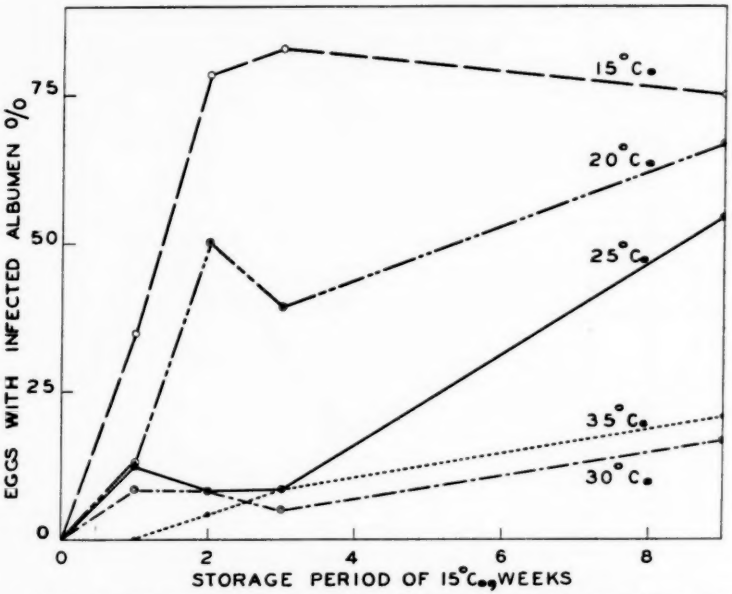

Figure 3. Relation of temperature differential between eggs and suspension to percentage infection. Each point represents the percentage of infected eggs in a 24-egg sample. Figures beside the curves denote the temperature of the suspension. All eggs were at 36°C.(96.8°F.) on immersion.

The results of repeatedly candling these eggs with the ultraviolet lamp are recorded graphically in Figure 4. In group A the rate of development of spoilage was essentially similar to that recorded in Figure 3 for the groups in which there was also little temperature differential. Subsequent sterile water immersion, however, resulted in much greater spoilage in group B, demonstrating that bacteria previously dried on the shell may subsequently penetrate to the albumen under suitable conditions. No fluorescent eggs developed in group C.

Infection of the Shell Membrane

Whenever eggs were broken for sampling in the experiments described above, portions of the shell membrane were stripped out and immersed in a

broth made of 0.1% asparagine, 0.05% $MgSO_4 \cdot 7H_2O$ and 0.05% K_2HPO_4 in distilled water. This medium enhances production of the pigment by fluorescent-pigment producing pseudomonads and is thus a sensitive indicator for these organisms *(3)*. When the data were analyzed, the unfortunate consequence of sampling only a portion instead of the whole membrane became apparent. The number of infected membrane samples always increased with the length of storage of the eggs. This increase suggests a

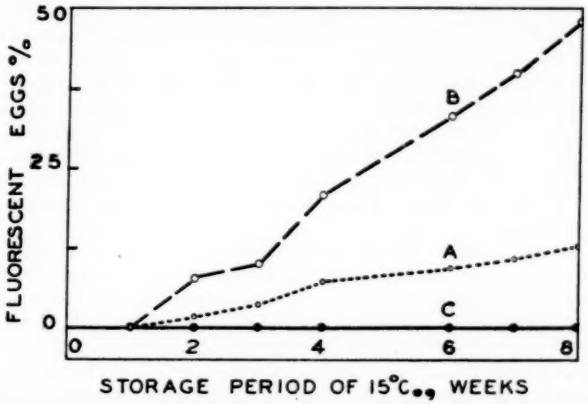

Figure 4. Development of fluorescent albumen in eggs due to infection with organisms previously dried on the shell, as determined by ultraviolet candling. For a description of lots A, B, and C, see text.

slow migration of organisms into the shell during storage, but might also be explained as a simple result of migration through the albumen. Shortly after albumen has become infected the entire membrane will also be infected; thus, infection in a membrane containing one or two colonies could readily be missed by the sampling technique, but once the colonies have spread to the albumen they would certainly not be missed.

Spreading of organisms was least in eggs broken out only 1 week after penetration. Using membrane data from these eggs only, high coefficients of correlation were obtained between the number of infected membrane samples and the number of fluorescent eggs ultimately found. The number of infected membranes in eggs broken at 1 week was almost exactly the same as the number of infected albumens found in eggs broken 9 weeks after penetration. These data indicate that the experimental variables described above affected the amount of egg spoilage merely by affecting the probability of penetration of organisms as far as the shell membrane. They also suggest that organisms lodged in the membrane may become the source of later infection of the albumen.

In the experiment on organisms dried on the shell, described above, a number of infected membranes were found, even in eggs from group C after 8 weeks of storage (eggs that had been immersed in sterile water only and in which no infected albumens developed). These infections must have

come from pseudomonads already present on the shell. Why they failed to infect the albumen was not determined.

Bird Individuality

Eggs from birds of several breeds (Single Comb White Leghorn, New Hampshire, New Hampshire-Plymouth Rock crosses, and Cornish) were collected and identified by the hen number. Every 7 days all sound eggs were subjected to the standard infection treatment except that the suspension contained only 10^3 organisms per ml. This dilution was expected to yield about 50% infected eggs after 6 weeks of storage (Figure 2); as a matter of fact, 52.2% of the 789 eggs so treated became infected after this period of storage. Eggs from different birds yielded strikingly divergent results, however. Of 55 birds laying 4 to 25 eggs, 4 produced eggs that all became infected, 4 produced eggs none of which became infected, and the eggs from the remaining birds had intermediate degrees of infection. The chi square value of 214 (based on calculated probabilities of infection) corresponds to an extraordinarily high probability that these differences were not due to chance.

The specific cause of the individuality observed here is not known. In these birds it was not related to shell thickness. Shell thickness ranged from 0.30 to 0.42 mm.; yet there was no correlation between shell thickness and percentage spoilage on a bird average basis, and no difference was found between average shell thickness of infected and non-infected eggs within individual birds. The characteristic number or size of pores may have played a role but this possibility was not investigated. Shell thickness may have an effect on probability of infection in extreme cases, however. Eggs were obtained from a flock that had recently recovered from an acute respiratory disease *(20)* and had, as a consequence, very thin, abnormal shells. These eggs were subjected to penetration with a dilute suspension which produced only 3.3% infection in normal eggs from other birds. The thin-shelled eggs became infected to the extent of 62.7%.

Probable Sources of Infection

The egg is moist when first laid, and is generally warmer than the immediate environment; thus infection from contaminated nests is at least a possibility. Pseudomonads similar to our strain include many soil organisms and might be expected to be present in nests, or at least, in unsanitary ones. Haines and Moran *(7)* failed to find evidence of infection in the nest, but data obtained here presents conclusive evidence that such infection can occur.

A quantity of wood shavings was contaminated by soaking in a suspension of the *Pseudomonas* strain. This material was air-dried for 5 days, and was then employed as nest litter in a small pen containing 8 hens. During the next 2 weeks 54 grossly clean sound eggs were laid. These eggs were collected twice daily and stored for 9 months at 15°C.; 64.8% were spoiled at the end of that time. Fluorescent spoilage was observed, but in many eggs the fluorescence was obscured by blackening of the contents. Meanwhile only 2.6% of the 117 eggs laid during the next month, and 9.4% of 159 eggs laid during the following 7 weeks, developed this infection.

Thus, contaminated nest litter, even if dry, can serve as a source of infection; however, the viability (or more properly the infectivity) of the organisms tends to decrease quite rapidly in the dry nest. Nevertheless, nest infection cannot be an important source of spoilage in the field, so long, at least, as the eggs are apparently clean. Of some 50,000 nest-clean and unwashed eggs with sound shells from various sources, examined in the authors' laboratory, less than 0.2% have shown evidence of *Pseudomonas* infection after storage. If the eggs are grossly soiled, the results may be quite different. In storage eggs from a single ranch, 8645 clean unwashed eggs had 0.21% spoilage; 1334 light dirties had 0.75%, and 2.10% of 617 unwashed heavy dirties spoiled *(17)*. On the other hand, a number of smaller samples from several other ranches had as little spoilage in light dirty as in clean eggs; 0.24% and 0.25%, respectively, for 3287 light dirties and 5088 cleans *(9)*. Apparently the masses of dirt that may stick to the shell of heavy dirties may hold enough organisms and keep the shell moist long enough to cause penetration in a significant number of instances, but the slight smudge or streak of a light dirty is only rarely more predisposing to bacterial penetration than the grossly clean shell.

Area of Shell

Many eggs become infected as a result of either contaminated mud or droppings at the time of laying or some time after they are laid, or both. Specific questions regarding this mode of infection include those concerning the effects of extent of the shell area soiled; of time interval after laying independent of temperature (that is, whether any change in the shell or shell pores as a result of the original drying might affect the egg's susceptibility to penetration); and of whether one area of the shell is more susceptible than another. These questions were explored in a limited experiment.

A sterile, autoclaved thin mud of mixed soil and droppings was heavily inoculated with the strain of *Pseudomonas* and stored in a refrigerator until used. Every other day eggs from a group of hens were collected as laid and immediately and momentarily dipped in the soil. Egg temperatures were approximately 30-35°C. and the soil was about 4°C. Some were completely immersed, others were half immersed, and still others were just touched to the mud surface so that an area of about 1 cm.^{2} was soiled. Some eggs were soiled at the large end and some at the small end. On alternate days eggs were collected from the same hens, stored overnight in an incubator at 30°C. and then treated as above. All eggs were dried in air and then stored for 8 weeks at 15°C.

TABLE 1

Infection of eggs with Pseudomonas-contaminated soil. (10 to 12 eggs per sub-group)

Area soiled	Position of soil	Time of soiling	
		Nest-warm eggs	Held eggs
		Infected %	*Infected* %
Complete	Entire egg	75.0	66.7
Half	Large end	25.0	29.2
Half	Small end	18.2	18.2
1 cm.^{2}	Large end	36.4	28.6
1 cm.^{2}	Small end	33.3	17.4

The numbers of infected eggs are recorded in Table 1. Complete immersion produced significantly greater infection than partial immersion (chi squared equals 16.2 with 2 degrees of freedom) but covering half the

shell was no more efficacious than soiling 1 cm.^{2} The difference in the number of infected eggs resulting from soiling the large ends as compared with the small ends of the eggs was not significant, nor was the difference in susceptibility to infection of just-laid and 24-hour old eggs.

SUMMARY

Experiments have been performed using an artificial infection technique consisting of immersing eggs for 5 minutes in suspensions of a single *Pseudomonas* strain which was isolated from a "fluorescent sour" egg. The probability of infection is increased, 1, by increasing the concentration of the suspension up to 10^6 cells per ml.; and 2, by increasing the temperature differential between the eggs and the suspension, when the eggs are the warmer, at least up to a differential of 21°C. Organisms previously dried on the shell were shown to be capable of causing infection when the shell was wetted under suitable conditions.

Eggs from the same birds become infected more readily than eggs from others. The difference is unrelated to shell thickness, although eggs with very thin abnormal shells, laid following a respiratory disease, were more susceptible to infection than normal eggs.

Eggs may be infected when laid in contaminated nests. If the shell is not obviously soiled, infection in the nest is rare. Eggs soiled in the nest become infected more frequently.

LITERATURE CITED

1. Bryant, R. L., and Sharp, P. F. Effect of washing on the keeping quality of hen's eggs. *J. Agr. Research*, **48**, 67 (1934).
2. Bushnell, L. D., and Mauker, O. Some factors influencing the bacterial content and keeping quality of eggs. Kansas Agr. Expt. Sta., *Bull.*, **201** (1914).
3. Clara, F. M. A comparative study of the green-fluorescent bacterial plant pathogens. N. Y. State Agr. Expt. Sta., *Memoir*, **159**, (1934).
4. Hadley, P. B., and Caldwell, D. W. The bacterial infection of fresh eggs. R. I. Agr. Expt. Sta., *Bull.*, **164** (1916).
5. Haines, R. B. Observations on the bacterial flora of the hens' egg with a description of new species of *Proteus* and *Pseudomonas* causing rots in eggs. *J. Hyg.*, **38**, 338 (1938).
6. Haines, R. B. Microbiology in the preservation of the hens' egg. Dept. Sci. Ind. Research, Food Invest., *Special Rep.*, **47** (1939).
7. Haines, R. B., and Moran, T. Porosity of and bacterial invasion through, the shell of the hens' egg. *J. Hyg.*, **40**, 453 (1940).
8. Lorenz, F. W., and Starr, P. B. Spoilage of washed eggs. I. Effect of sprayed vs. static water under different washing temperatures. *Poultry Sci.*, **31**, 204 (1952).
9. Lorenz, F. W., Ogasawara, F. X., and Starr, P. B. Spoilage of washed eggs. III. A survey of ranch practices and results. *Poultry Sci.*, **31**, 221 (1952).
10. Lorenz, F. W. The use of ultraviolet light in egg-candling equipment. (Paper in preparation.)
11. Mallman, W. S., and Davidson, J. A. Oil-protected shell eggs. *U. S. Egg Poultry Mag.*, **50**, 113 (1944).
12. Miles, A. A., and Halnan, E. T. A new species of microorganism (*Proteus melanovogenes*) causing black rot in eggs. *J. Hyg.*, **37**, 79 (1937).
13. Pennington, M. E. A chemical and bacteriological study of fresh eggs. *J. Biol. Chem.*, **7**, 109 (1910).
14. Pennington, M. E., Jenkins, M. K., St. John, E. Q., and Hicks, W. B. A bacteriological and chemical study of commercial eggs in the producing districts of the central west. U. S. Dept. Agr., *Bull.*, **51** (1914).
15. Rettger, L. F. The bacteriology of the hens' egg with special reference to its freedom f om microbic invasion. Univ. Conn. Agr. Expt. Sta., *Bull.*, **75**, (1913).
16. Sharp, P. F., and Whitaker, R. The relation of the hydrogen ion concentration of egg white to its germicidal action. *J. Bact.*, **14**, 17 (1927).
17. Starr, P. B., Lorenz, F. W., and Ogasawara, F. X. Spoilage of washed eggs. II. Laboratory vs. ranch washing. *Poultry Sci.*, **31**, 215 (1952).
18. Starr, P. B., Starr, M. P., and Lorenz, F. W. The development of *Pseudomonas* spoilage in shell eggs. II. The bacteria which cause "fluorescent-sour" eggs. (Paper in preparation.)
19. Stuart, L. S., and McNally, E. H. Bacteriological studies on the egg shell. *U. S. Egg Poultry Mag.*, **49**, 28 (1943).
20. Taylor, L. W., Gunns, C. A., Grau, C. R., and Lepkovsky, S. Effect of a respiratory disease on reproduction. *Poultry Sci.*, **29**, 782 (1950).

EFFECT OF TEMPERATURE ON CERTAIN PRODUCTS OF VINOUS FERMENTATION

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Although in the fermentation of fruit juices by wine yeast, *Saccharomyces cerevisiae* var. *ellipsoideus*, the principal products formed are ethyl alcohol and carbon dioxide, certain other products of fermentation are of considerable importance, since they affect the flavor and bouquet of the wine. Enologists and wine makers are of the opinion that wines made by fermentation at fairly low temperatures are of better quality than those fermented at relatively high temperatures, owing, it has been thought, to the production of larger amounts of the desirable minor constituents of alcoholic fermentation and smaller amounts of undesirable constituents.

Some of these minor constituents are glycerol, succinic acid, acetaldehyde, acetic acid, lactic acid, and esters. Although higher alcohols are also produced, Ehrlich *(5)* has reported that they are formed by the action of yeast on certain amino acids.

According to modern theory, alcoholic fermentation proceeds step-wise with the formation of several intermediate products. Very briefly, the main steps are considered to be: phosphorylation of hexose sugar to a hexose-phosphate; breaking down of the hexose phosphate to two triose phosphates; dephosphorylation of the latter, and formation from the triose phosphates of ethyl alcohol and of CO_2 as the chief end products *(3)*.

Glycerol is considered to be a desirable constituent of wine, as it imparts smoothness or "fullness" to the flavor. Acetic acid, the chief constituent of the volatile acidity of wine, is considered undesirable in its effect on flavor and is an index of soundness of wine, since a high volatile acidity is an indication of bacterial activity.

Acetaldehyde, according to Amerine and Joslyn *(2)* is considered an undesirable constituent because of its adverse effect on flavor, color, and stability.

The small amounts of lactic acid formed during alcoholic fermentation probably do not greatly influence the flavor or bouquet of the wine. Under favorable conditions rather large amounts of lactic acid can be formed in wine by the so-called malo-lactic bacteria which transform malic acid to lactic acid by removal of one CO_2H group per molecule, thereby reducing the titratable acidity and the "tartness" of the wine as discussed by Suverkrop and Tchelitscheff *(13)* and others. In our experiments the malo-lactic fermentation was prevented by the use of pure yeast cultures and sterile juice.

Numerous esters are found in wines according to Peynaud *(11)*. These include neutral esters, and acid esters, both of which may be either volatile or fixed. Some of these favorably affect flavor and bouquet, whereas ethyl

acetate in appreciable concentration affects them adversely as it imparts an "acetic" odor and taste.

EXPERIMENTAL METHODS

Fermentations. In several experiments, commercial grape concentrate was diluted to 23° Brix, and sterilized in live steam in 500-ml. portions in bottles plugged with cotton. After cooling, the juices were inoculated with pure cultures of wine yeast.

In the first series of fermentations, temperatures of 36.6°C.(98°F.), and 32.3°C. (90°F.) were used. Fermentation was so slow at 6.6°C. that this temperature was not used in most cases and at 36.6°C. the fermentations failed to go to completion. In most of our experiments, therefore, three temperatures only were used, namely 30°C. to 32.3° C.(86-90°F.), 18.3° to 23.9°C.(65-75°F.), and 12.8°C.(55°F.).

Fresh juice was used in several experiments. It was not sterilized by heating, but instead, SO_2 was added in sufficient amount to prevent growth of undesired wild yeasts but not retard the activity of the added wine yeast. In one experiment, pasteurized Concord variety juice was used and in another fresh Boysenberry juice, sweetened to 23° Brix with dextrose.

The progress of fermentation was followed in most cases by taking the Brix degree of the juice once a day.

After fermentation had ended the fermented juices were allowed to settle a few days in the cold room at near 0°C.; were then drawn off from the sediment; bottled, and stored at −18.8°C.(0°F.) in full, sealed bottles until analyzed.

After fermentation had ceased, the fermented juices were examined microscopically, and were plated on nutrient agar in order to determine whether lactic or acetic bacteria were present. None was found.

Methods of analysis. Sugar was determined by the ceric sulfate method of Hassid *(7)* in the dealcoholized sample clarified with neutral lead acetate and deleaded with Na_2HPO_4.

Alcohol was determined by the dichromate oxidation method of Zimmerman and Fessler *(15)*.

Glycerol was determined by the procedure described by Amerine and Dietrich *(1)*.

Volatile acid was determined by the customary steam distillation procedure as described by Joslyn and Amerine *(3)* and acetaldehyde by the method described by the same authors *(10)*.

Neutral volatile esters were measured by determining the amount of 0.1 *N*. NaOH required to saponify esters in the distillate from the wine as outlined by Joslyn and Amerine *(10)*.

According to Amerine[a] none of the published methods for determining lactic acid are entirely satisfactory but the permanganate procedures of Friedmann and Graesaer *(6)* and of Peynaud *(12)* are commonly used in this country, and give comparable results. We used Peynaud's method since the necessary equipment was on hand.

Tabulation of data. The more significant of our results has been presented in Table 1. The data are given both in terms of weight per 100 ml. and of weight of the minor constituents formed per gram of sugar fermented. When fresh grapes were used, they were crushed and pressed and to the juice was added 150 p.p.m. of SO_2 in the form of potassium metabisulfite. Lots of 2,000 ml. of the juice in duplicate were fermented with "Burgundy" wine yeast (University of California culture No. 51), at 3 different temperatures: 31.2°C.(88°F.), 18.3-23.9°C.(65-75°F.), and 12.8°C.(55°F.). The juice before fermentation was of the following composition: Brix degree, 23.25; total acid as tartaric, 0.561 gram per 100 ml.; and volatile acid, 0.005 gram per 100 ml. (pH value, 3.47).

When concentrates were used, they were diluted with water to 23° Brix and sterilized in bottles at 100°C. After cooling overnight or longer the juice was inoculated with a pure culture of wine yeast and lots in duplicate were fermented at the temperatures shown in the table. The values shown are averages of duplicates. Alcohol content of the resulting wines is not shown in the table since it is a major, not a minor constituent. It tended to be higher at the lower fermentation temperatures, probably because of greater

[a] Personal communication.

TABLE 1

Glycerol, lactic acid, volatile acid, neutral esters and aldehyde produced at several different temperatures of vinous fermentation

Temperature	Raw Material	Glycerol		Lactic acid		Volatile acid		Neutral esters		Aldehyde	
		Per 100 ml.	Per g. sugar fermented	Per 100 ml.	Per g. sugar fermented	Per 100 ml.	Per g. sugar fermented	Per 100 ml.	Per g. sugar fermented	Per liter	Per g. sugar fermented
° C.		g.	g.	g.	g.	g.	g.	mg.	mg.	mg.	mg.
36.6	Dilute	0.764	0.063	.....	.....	0.095	0.0062	7.04	0.585	74.2	0.616
32.3	white	0.909	0.046	.....	.....	0.099	0.0039	7.92	0.397	60.1	0.302
18.3-23.9	concen-	0.706	0.031	0.111	0.0049	0.092	0.0032	5.81	0.259	71.1	0.317
12.8	trate	0.698	0.031	0.064	0.0028	0.101	0.0035	8.10	0.356	93.9	0.412
6.6		0.782	0.049	0.086	0.0054	0.129	0.0068	16.50	1.04	60.3	0.380
36.6	Dilute	0.664	0.047	.....	.....	0.064	0.0031	2.99	0.214	64.0	0.457
32.3	red	0.835	0.044	.....	.....	0.070	0.0026	6.69	0.352	46.0	0.242
18.3-23.9	concen-	0.743	0.033	0.101	0.0044	0.094	0.0032	5.46	0.240	70.0	0.309
12.8	trate	0.769	0.034	0.075	0.0033	0.106	0.0038	6.34	0.281	50.6	0.224
6.6		0.778	0.043	0.045	0.0025	0.080	0.0033	10.20	0.569	69.1	0.386
30	Concord	0.715	0.036	0.086	0.0043	0.042	0.0016	21.2	1.065	37.4	0.188
18.3-23.9	juice	0.730	0.034	0.077	0.0035	0.029	0.00087	28.8	1.320	27.6	0.127
12.8		0.688	0.036	0.077	0.0040	0.016	0.00031	19.4	1.010	9.6	0.050
30.8	Dilute	0.986	0.045	.....	.....	0.037	0.0022	7.05	0.324	53.3	0.244
12.8	Alicante	0.901	0.042	.....	.....	0.048	0.0058	14.10	0.650	43.6	0.202
6.6	concentrate	0.908	0.055	.....	.....	0.096	0.0015	21.10	1.280	59.8	0.364
31.2	Fresh	0.963	0.043	.....	.....	0.035	0.00125	15.8	0.710	50.8	0.226
18.3-23.9	Alicante	0.835	0.037	.....	.....	0.032	0.00111	19.2	0.855	45.8	0.204
12.8	juice	0.688	0.031	.....	.....	0.040	0.00147	15.8	0.710	38.7	0.172
32.2	Boysen-	0.809	0.040	0.063	0.0031	0.016	0.000297	60.8	3.54	54.5	0.269
18.3-23.9	berry	0.865	0.043	0.059	0.0029	0.016	0.000297	63.4	3.79	43.1	0.213
12.8	juice	0.862	0.043	0.067	0.0033	0.021	0.000545	57.8	3.36	39.4	0.195
31.2	Fresh	.....	.....	.....	.....	0.040	0.0015	26.4	1.165	89.5	0.390
18.3-23.9	Thompson	.....	.....	.....	.....	0.040	0.0015	22.9	1.010	78.7	0.342
12.8	juice	.....	.....	.....	.....	0.047	0.0018	27.3	1.200	73	0.318

loss of alcohol by volatilization at the higher temperatures. In several experiments the volatilized alcohol was entrapped and determined.

RESULTS

In a typical experiment with fresh juice the alcohol contents of the fermented liquids were: at 31.2°C., 10.37 g. per 100 ml.; at 18.3-23.9°C., 10.57 g. per 100 ml.; and at 12.8°C., 10.65 g. per 100 ml. The residual unfermented sugar was 0.20 g. per 100 ml. in the three cases. The original juice was 23° Brix.

In one experiment pure cultures of two different wine yeasts were compared, namely Burgundy No. 51, and California No. 9; but they showed very little difference in respect to amounts of minor constituents formed.

Glycerol. Production of glycerol per gram of sugar utilized was higher at the lowest fermentation temperatures in only two cases, equal in one, and slightly lower in five experiments, indicating a trend toward production of slightly less glycerol per unit of sugar fermented at the lower temperatures. In most instances the difference was not great. See Columns 3 and 4 in the table.

Our results are somewhat contrary to those reported by Tchelitscheff *(14)* in 1948 in winery scale fermentations, of Hickinbotham and Ryan *(8)* in 1948, and of Brockmann and Stier *(4)* in laboratory scale experiments. However, Tchelitscheff *(14)* did not use tanks of similar size in his comparative tests and in one case a closed fermenter was used and in the other an open one.

Hickinbotham and Ryan *(8)* used small volumes of juice (272 ml.) in cotton stoppered bottles. Consequently oxidative conditions might have prevailed. The difference in temperatures used by Brockmann and Stier *(4)* was slight (3°C. difference, 37° versus 40°C.), and were above the optimum for most yeasts. Also, they used continuous agitation during fermentation. It would seem that under such conditions yeast respiration might compete with fermentation. At any rate, their conditions were not at all comparable to those used by us.

Total acid. Total acidity of the wines fermented at low temperatures tended to be slightly lower than of those fermented at higher temperatures, owing probably to the separation of a larger amount of cream of tartar at the lower temperatures. The data has not been presented in the table since it is likely that the yeast does not attack either malic or tartaric acid.

Lactic acid. Lactic acid was not determined in all experiments since none of the available methods for determination of this acid in wines is very dependable. However, for what they may be worth, the results obtained for lactic acid are given in Columns 5 and 6 of Table 1.

With the three grape juices used, lactic acid production tended to be somewhat less at the lower temperatures of fermentation but was not materially affected by the temperature in the case of Boysenberry juice.

Volatile acid. It is common belief among wine producers that under practical winery conditions volatile acid formation is usually higher at excessively high temperatures of fermentation than at relatively low temperatures, because of the danger of growth of lactic acid bacteria and acetic bacteria. In our experiments the activity of such bacteria was prevented,

as previously outlined. Columns 7 and 8 summarize the data obtained in our experiments. We have expressed the results both in grams per 100 ml. of wine and as grams of volatile acid per gram of sugar utilized, as wine chemists are more accustomed to the former method of reporting.

On the basis of grams of volatile acid formed per gram of sugar utilized, there appeared to be a tendency for somewhat greater production of volatile acid at the lowest temperature used. Since fermentation is extremely slow at such low temperatures, it is possible that the yeast responds somewhat abnormally in respect to volatile acid production. However, the differences were in most cases not very large. The diluted white concentrate possessed considerable volatile acid before fermentation which accounts in part for the abnormally high volatile acidity of the resulting wines in this case.

Acetaldehyde. In 6 experiments acetaldehyde production in mg. per gram of sugar utilized was lower at the lowest fermentation temperature than at the highest temperatures of fermentation, and in one case it was somewhat higher, indicating that the trend is toward lower production of acetaldehyde at the lower temperatures of fermentation. As high aldehyde content is considered to be undesirable in dry wines, this trend is desirable. Since acetaldehyde can be formed in wines by oxidation by air, even in the absence of yeast it is possible that some of the aldehyde in our experiments may have been formed in such manner.

Neutral esters. The neutral ester content of the experimental wines and the corresponding production of neutral esters per gram of sugar utliized are given in Columns 9 and 10 in Table 1. In four experiments there was somewhat greater production of neutral esters per gram of sugar fermented at the lowest temperature used than at the highest temperatures; in one case there was no difference; and in two cases it was slightly lower at the lowest temperature of fermentation. Consequently, in our experiments neutral ester production tended to be slightly higher at the lower temperatures of fermentation. The fermented loganberry juice showed a relatively high neutral ester content in all three samples, due perhaps to esters occurring in the fresh fruit.

SUMMARY

The investigation reported in this paper was for the purpose of obtaining data on the effect of temperature of fermentation by *Saccharomyces cerevisiae* variety *ellipsoideus* (wine yeast) on several minor products of fermentation.

In our experiments the trend was toward the production of slightly less glycerol at the lower temperatures of fermentation. In the 4 experiments in which lactic acid content of the fermented juices was determined, there was on the average slightly less lactic acid formed at the lower temperatures. There appeared to be no consistent correlation between temperature of fermentation and production of volatile acidity. Production of aldehyde was lower at the lower temperatures of fermentation. In the majority of our experiments neutral ester production was slightly higher on the average at the lower temperatures of fermentation.

Alcohol content of the fermented juices was higher on the average at the lower temperatures of fermentation, owing to loss of entrainment and volatilization at the higher temperatures.

Total acidity was on the average slightly lower in the wines fermented at the lower temperatures, owing probably to more extensive loss of acid potassium tartrate (cream of tartar) by crystallization at the lowest temperatures used, since its solubility varies with temperature.

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LITERATURE CITED

1. AMERINE, M. A., AND DIETRICH, W. C. Glycerol in wines. *J. Assoc. Offic. Agr. Chemists*, **26**, 408 (1943).
2. AMERINE, M. A. AND JOSLYN, M. A. Commercial production of table wines. Univ. Calif. Agr. Expt. Sta., *Bull.* **639** (1940). (See also same authors' Table Wines. Univ. Calif. Press, 1951.)
3. BALDWIN, E. *Dynamic Aspects of Biochemistry*, 1948, Cambridge University Press, England.
4. BROCKMANN, M. C., AND STIER, T. J. B. Influence of temperature on the production of glycerol during alcoholic fermentation. *J. Am. Chem. Soc.*, **70**, 413 (1948).
5. EHRLICH, F. Zur Frage der Fuselölbildung Hefe. *Ber.*, **39**, 4072 (1906).
6. FRIEDMAN, T. E., AND GRAESER, J. B. The determination of lactic acid. *J. Biol. Chem.*, **100**, 291 (1933).
7. HASSID, W. Z. Determination of sugar in plants. *Ind. Eng. Chem., Anal. Ed.*, **9**, 228 (1937).
8. HICKINBOTHAM, A. R., AND RYAN, V. J. Glycerol in wine. *Australian Chem. Inst. J. and Proc.*, **3**, 89 (1948).
9. JOSLYN, M. A., AND AMERINE, M. A. Commercial production of dessert wines. Univ. of Calif. Agr. Expt. Sta., *Bull.*, **651** (1941).
10. JOSLYN, M. A., AND AMERINE, M. A. Commercial production of brandies. Univ. Calif. Agr. Expt. Sta., *Bull.*, **652** (1941).
11. PEYNAUD, E. L'acetate d'ethyl dans les vins atteints d'acescence. *Ann. fermentations*, **2**, 367 (1936).
12. PEYNAUD, E. Etude des methodes de dosage des constituants de l'acidite de vins. *Chimie Analytique*, **4**, **28**, 127 (1946).
13. SUVERKROP, B., AND TCHELITSCHEFF, A. Comments on malo-lactic fermentations in Napa Valley wines. *Wines and Vines*, **30** (7), 197 (1949).
14. TCHELITSCHEFF, A. Researches on cool fermentation. *Wines and Vines*, **29** (9), 23 (1948).
15. ZIMMERMAN, H., AND FESSLER, J. Chemical method for alcohol determination. *Wines and Vines*, **30** (9), 65 (1949).

ASCORBIC ACID IN TOCOPHEROL-ENRICHED MILK[a]

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The amount of ascorbic acid in commercial milk is exceedingly variable due to several influencing factors. Furthermore the rate of decrease of the ascorbic acid in market milk is not uniform. Even after milk has reached the average home it rapidly loses a large portion of its ascorbic acid. Thus the amount of ascorbic acid that a person obtains from commercial fluid milk is unknown and extremely variable. Moreover this situation exists with respect to other vitamins that are present in milk. Investigators have found the following amounts of tocopherol per 100 g. of cows' milk fat: Harris, Swanson, and Hickman *(1)*, 4200 γ for summer milk fat and 2300 γ for winter milk fat; Loosli *(7)*, 2087 γ for winter and 2736 γ for pasture milk fat; Krukovsky, Whiting, and Loosli *(5)*, 3033 γ for Guernsey and 2220 γ for Holstein milk fat; Krukovsky, Loosli, and Whiting *(6)*, 1873 γ for milk fat from cows fed ladino clover hay and late cut timothy hay but cows fed birds-foot trefoil hay produced milk that contained 2952 γ of tocopherol per 100 g. of milk fat. Whiting, Loosli, Krukovsky, and Turk *(9)* stated that the tocopherol content of milk fat was increased by feeding tocopherols. Krukovsky, Loosli, and Whiting *(5)* also reported that a significant correlation was found between the tocopherol content of milk fat and the ability of milk to resist the reaction, involving ascorbic acid oxidation, which produces oxidized flavors. These observations raised a question regarding the stability of ascorbic acid in tocopherol-enriched milk; this study was undertaken to answer that question.

EXPERIMENTAL

The University Department of Dairy Industry supplied the milk tested in this study: part was produced by the university herd of purebred Ayrshire, Guernsey, Holstein, Jersey, and Shorthorn cows, and part was purchased from commercial sources. The university and commercial milks were thoroughly mixed before pasteurization. As soon as the milk was pasteurized and cooled to 40°F.(4.5°C.), it was taken to the laboratory. The milk was divided into 500-ml. portions which were placed in quart, flint glass commercial milk bottles. Since the bottles were about half filled the milk was in contact with air as frequently occurs in a normal household. One bottle of milk was retained as a control. Alpha tocopherol was added to the milk in the other bottles at the rate of 0.2, 0.4, 0.8, 1.2, 1.6, 2.0, and 2.4 mg. per liter. The tocopherol was dissolved in ether. Two solutions were prepared: one contained 1 mg. and the second 2 mg. of tocopherol per ml. A sufficient amount of one or the other of these solutions was added per liter of milk to produce a series of samples of enriched milk that contained the amount of tocopherol previously mentioned. As soon as the samples were prepared they were thoroughly shaken to disperse the ether solution throughout the milk and the samples were shaken again just before the 25-ml. aliquots were withdrawn for assay.

During the storage period samples of control milk and tocopherol-enriched milk were kept in darkness at 50°F.(10°C.). A series of 16 samples was prepared on Mondays at regular intervals during the academic year. The samples were assayed for

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reduced ascorbic acid as soon as they were prepared and also at 4 subsequent 24-hour periods. Thus the experimental period exceeded somewhat the length of time during which milk is ordinarily stored in the average household refrigerator. The Sharp *(8)* rapid method for the quantitative determination of reduced ascorbic acid in milk as modified by Holmes *(2)* was followed in assaying the milk tested in this study.

RESULTS AND DISCUSSION

The average amounts of reduced ascorbic acid, Table 1, in the untreated milk at the 5 assay periods, namely 17.1, 11.2, 7.1, 5.2, and 4.7 mg. per liter show that the milk lost 34.5, 24.0, 11.1, and 2.9%, respectively, during the 4 successive 24-hour storage periods or a total of 72.5 during the 96 hours

TABLE 1

Decrease of reduced ascorbic acid in alpha-tocopherol-enriched milk during storage in darkness at 10°C.

Amount of alpha-tocopherol added to the milk	Amount of reduced ascorbic acid in milk									
	1st day	2nd day		3rd day		4th day		5th day		
mg. per liter	*mg. per liter*	*mg. per liter*	*% loss*	*mg. per liter*	*% loss*	*mg. per liter*	*% loss*	*mg. per liter*	*% loss*	*% total loss*
0.0	17.1	11.2	34.5	7.1	24.0	5.2	11.1	4.7	2.9	72.5
0.2	17.1	11.1	35.1	6.8	25.1	5.6	7.0	4.7	5.3	72.5
0.4	17.1	11.8	31.0	7.1	27.4	5.5	9.4	4.6	5.3	73.1
0.8	17.1	12.0	29.8	7.1	28.7	5.9	7.0	4.8	6.4	71.9
1.2	17.1	11.4	33.3	7.4	23.4	5.8	9.4	5.2	3.5	69.6
1.6	17.1	11.2	34.5	7.3	22.8	5.7	9.4	5.3	2.3	69.0
2.0	17.1	11.2	34.5	7.5	21.6	6.0	8.8	5.3	4.1	69.0
2.4	17.1	11.6	32.2	7.7	22.8	5.8	11.1	5.4	2.3	68.4

of storage. These losses of reduced ascorbic acid from pasteurized milk stored in darkness at 50°F.(10°C.) are quite similar to losses of 31, 23, 14, and 9% with a total loss of 77% reported by Holmes *(3)* for milk stored under the same controlled conditions. In another study, conducted from April to August, in which a series of samples of commercial pasteurized milk was stored for 96 hours in darkness at 50°F.(10°C.) Holmes *(4)* observed reduced ascorbic acid losses of 41.5, 21.1, 7.5, and 4.1%, respectively, for four 24-hour periods with a total loss of 74.2% for 96 hours of continuous storage.

The results obtained during the 16-week experimental period from 640 assays of the 8 series of milk samples are summarized in Table 1. The 7 series of milk samples to which 0.2, 0.4, 0.8, 1.2, 1.6, 2.0, and 2.4 mg. of alpha tocopherol per liter were added, and the control samples all had an average initial reduced ascorbic acid content of 17.1 mg. per liter.

The loss of ascorbic acid from the 7 series of tocopherol-enriched milk samples varied from 29.8% to 35.1% during the first 24 hours of storage, from 21.6% to 28.7% during the second storage period, from 7.0% to 11.1% during the third 24-hour period, and from 2.3% to 6.4% during the final storage period. The average total loss of reduced ascorbic acid during 96 hours of storage, from the 7 series of samples of alpha tocopherol enriched milk was 72.5, 73.1, 71.9, 69.6, 69.0, and 68.4%, respectively, as

compared with a loss of 72.5% reduced ascorbic acid from the control sample. In other words the samples of milk enriched with from 0.2 to 2.4 mg. of alpha tocopherol per liter lost as much reduced ascorbic acid as the control samples. Even though the loss of reduced ascorbic acid decreased fairly consistently with the addition of increasing amounts of alpha tocopherol, the amount of the decrease was not commercially significant. In fact, judged by the results obtained here, enriching milk with alpha tocopherol does not enhance the stability of the reduced ascorbic acid sufficiently to justify its use on a commercial scale.

SUMMARY

It has been reported that feeding cows tocopherol enhances the keeping quality of their milk. This study was undertaken to test the feasibility of adding tocopherol to commercial milk. Mixed herd milk was enriched with 0.2, 0.4, 0.8, 1.2, 1.6, 2.0, and 2.4 mg. of alpha tocopherol per liter. A sample of milk containing each of the 7 amounts of tocopherol and a control sample were prepared on 16 Monday mornings spaced throughout the academic year. All the samples were stored in darkness at 50°F.(10°C.) for 96 hours. When the samples were prepared and at 4 subsequent 24-hour periods they were assayed for reduced ascorbic acid. The addition of alpha tocopherol to milk, particularly the larger amounts, decreased somewhat the loss of reduced ascorbic acid from milk during storage but not sufficiently to justify its use on a commercial scale.

LITERATURE CITED

1. Harris, P. L., Swanson, W. J., and Hickman, K. C. D. Covitamin studies. VI. Effect of tocopherol supplementation on the output of vitamin A, carotene, and fat by dairy cows. *J. Nutrition*, **33**, 411 (1947).
2. Holmes, A. D. Rate of destruction of reduced ascorbic acid in riboflavin fortified pasteurized milk. *Food Technol.*, **4**, 92 (1950).
3. Holmes, A. D. Comparison of the stability of reduced ascorbic acid in raw and pasteurized milk. *Am. J. Diseases Children*, **78**, 899 (1949).
4. Holmes, A. D. Loss of reduced ascorbic acid from lactose-enriched milk. *J. Dairy Sci.*, **32**, 556 (1949).
5. Krukovsky, V. N., Whiting, F., and Loosli, J. K. Tocopherol, carotenoid and vitamin A content of the milk fat and the resistance of milk to the development of oxidized flavors as influenced by breed and season. *J. Dairy Sci.*, **33**, 791 (1950).
6. Krukovsky, V. N., Loosli, J. K., and Whiting, F. The influence of tocopherols and cod liver oil on the stability of milk. *J. Dairy Sci.*, **32**, 196 (1949).
7. Loosli, J. K. Vitamin E requirement and economy in farm animals. *Ann. N. Y. Acad. Sci.*, **52**, 243 (1949).
8. Sharp, P. F. Rapid method for the quantitative determination of reduced ascorbic acid in milk. *J. Dairy Sci.*, **21**, 85 (1938).
9. Whiting, F., Loosli, J. K., Krukovsky, V. N., and Turk, K. L. The influence of tocopherols and cod-liver oil on milk and fat production. *J. Dairy Sci.*, **32**, 133 (1949).

FACTORS WHICH CONDITION FIRMNESS IN TOMATOES [a]

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A new source of genetic variability shows promise of affording plant breeders an opportunity of developing firm varieties of tomatoes which may be valuable for both fresh market and canning. The parent material from which a number of firm lines of tomatoes have been developed resulted from the cross *(Lycopersicon esculentum* × *L. pimpinellifolium)* × *(L. esculentum* × *L. peruvianum)* which was made by Dr. O. H. Pearson of the Eastern States Farmers Exchange, Springfield, Mass.

Several of the firm fruited lines produce tomatoes which are firmer when red-ripe than "turning" fruits of the soft fruited varieties, Valiant and Waltham Forcing. Firmness was measured by means of a pressure tester which measures the compression of a fruit supporting a 1-inch plunger on which is placed a 2-kg. weight for 5 seconds *(9)*. The marked differences in firmness appeared to be due, at least in part, to a much greater content of fibrous core in fruits of some firm lines; however, other firm lines produced fruits which contained very little fibrous core and were equally firm. Pressure tests on 1-inch slices of inherently firm and soft fruits of equal maturity indicated marked differences in the firmness of the fleshy tissue. The study reported here was conducted to determine the extent to which differences in firmness could be explained by differences in the contents of calcium and pectic substances between firm and soft tomatoes.

REVIEW OF LITERATURE

The calcium content of plant tissues was shown by Kertesz *(14)* to have an effect on firmness. He found that tomatoes contain a very active pectinmethylesterase enzyme which demethylates pectin. Since it was known that calcium combines with demethylated pectin to form a gel, he surmised that the addition of small amounts of calcium to tomato tissue would result in the formation of a calcium pectate gel in the tissue which would provide additional support to the cell structure resulting in increased firmness. Later Loconti and Kertesz *(18)* definitely showed that this assumed relationship was valid.

Since additions of calcium to tomatoes resulted in a firming of the tissue, it seems reasonable to assume that an inherent ability of tomatoes to accumulate more calcium might have a similar effect. This possibility seems important because of the very low content of calcium in the commercial varieties of tomatoes. Smith *(23)*, Harvey *(10)*, and Weiss *(24)* noted a differential efficiency of various strains of corn, tomatoes, and soybeans in accumulating mineral elements.

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Sayre, Kertesz, and Loconti *(21)* found that the addition of calcium to the soil in which Nystate tomatoes were grown had no appreciable effect on the calcium content or firmness of the tomatoes. Beeson *(3)* found that the calcium content of tomatoes increased with the calcium supplied to the nutrient culture, but he did not measure the relation between calcium content and firmness.

Besides forming a calcium-pectate gel which adds to the firmness of tomato tissue, pectic substances in the middle lamellae cement adjacent cells together and, because of their pliability, are very important in adding firmness and rigidity to the tissue during the period of rapid growth and expansion *(1)*.

The content of protopectin, or water-insoluble pectin, of the middle lamellae of many fruits was found to decrease as the fruits mature *(2, 4, 6, 7, 8, 12, 17)*. This change results in softening and finally maceration of the tissue.

According to Kertesz and Loconti *(16)* there is considerable variation in the total pectic content and the characteristics of pectic substances in different tomato varieties, as had also been observed by Saywell and Cruess *(22)* and Kassab *(13)*.

EXPERIMENTAL METHODS

Pot experiment. A pot experiment was conducted in the greenhouse to measure the relative absorption of calcium by 4 firm lines; 2 lines showing intermediate firmness, derived from second backcrosses to soft varieties, and the varieties Valiant and Waltham Forcing. Plants were grown in 12-inch pots containing well mixed soil which was found by soil test to have a high calcium content, 21,000 pounds of calcium per acre (2,000,000 pounds of soil), mostly as calcium carbonate. The insides of the pots were painted with 2 coats of chemically inert Bass Heuter asphalt varnish, and to prevent the plants from obtaining calcium except from the soil in the pots, the pots were set in the greenhouse on 2-inch wooden rails. The 8 replicates of the 8 varieties were grown on trellis in a latin square with a single plant to a replicate.

Nutrient experiment. A comparison was made between a firm line 49-774-4 (F_4) and Valiant, with one plant of each grown in the same tank, with nutrient solutions of low, medium, and high contents of calcium replicated 6 times. Low, medium, and high levels of boron were used with the corresponding levels of calcium to maintain a suitable relationship between calcium and boron. The plants were grown in 15-liter cast iron tanks painted inside with two coats of Bass Heuter asphalt varnish and provided with constant aeration by means of perforated aluminum aerators. They were covered by aluminum tops also painted with asphalt varnish. The plants were held in place by means of non-absorbent cotton and tied to a trellis above the tanks.

Hoagland's No. 2 *(11)* was used as the basic nutrient solution which was altered to contain 2, 8, and 14 milliequivalents of calcium with corresponding contents of 0.01, 0.5, and 1.0 p.p.m. of boron. The nutrients used for the three levels of calcium are shown in Table 1. The average pH for the solutions was 6.67 for low, 7.14 for intermediate, and 7.32 for high levels of calcium and boron.

Seedlings were grown in quartz sand and in the nutrient tanks containing Hoagland's No. 2 until the second cluster of fruit had set, after which the three nutrient levels were applied. The low level of boron was not sufficient to sustain growth so applications of 1 ml. per liter of a 0.01 p.p.m. solution of boron were made every 3 days. The nutrient solutions were changed at intervals of 2 weeks.

Preparation of samples. The first tomatoes which ripened on the plants grown in pots and in nutrient solutions were weighed in two samples of approximately 100 g. each. The seeds and gelatinous material were removed; so the samples were composed of fleshy material and skin. These samples were dried in silica dishes at 70°C. for 72 hours in a forced draft oven and ashed in a muffle furnace at 450-500°C. for 16 hours. Ten ml. of concentrated nitric acid was added and evaporated to dryness on a

hot plate, after which the samples were heated for 1 hour in the muffle furnace. After cooling, 50 ml. of 1 to 10 hydrochloric acid was added, and the dishes were covered with watch glasses and digested on a hot plate with low heat until the ash was dissolved. The samples were then diluted to volume in a 100-ml. volumetric flask, after which they were kept in capped bottles until analyzed.

Calcium determinations. Calcium determinations were made by the semi-micro method developed by Reitemeier *(20)* for the analysis of soil solutions and plant extracts when the available sample is small or the concentration of calcium is low.

The firming effect of calcium chloride on fresh tomato tissue. The 6 firm selections, Valiant, and Waltham Forcing were compared in a test to determine the relative firming effect of two solutions of calcium chloride in which 1-inch slices of the tomatoes were immersed for different lengths of time. The calcium chloride treatments consisted of: 1, a 5-minute immersion in a 5% solution; 2, 30 minutes' immersion in a 5% solution; and 3, 12 hours' immersion in a 2.5% solution of calcium chloride. The firmness of slices from the three treatments was compared to similar untreated slices by means of a pressure tester using a 1-inch plunger and a 2-kg. weight.

TABLE 1

Nutrients used for three levels of calcium in nutriculture experiment

Low calcium		Intermediate calcium		High calcium	
Nutrient	ml. of M. solution per liter distilled water	Nutrient	ml. of M. solution per liter distilled water	Nutrient	ml. of M. solution per liter distilled water
$NH_4H_2PO_4$	1	$NH_4H_2PO_4$	1	$NH_4H_2PO_4$	1
KNO_3	6	KNO_3	6	KNO_3	6
$Ca(NO_3)_2$	1	$Ca(NO_3)_2$	4	$Ca(NO_3)_2$	4
$MgSO_4$	2	$MgSO_4$	2	$MgSO_4$	2
$NaNO_3$	6	NaCl	6	$CaCl_2$	3

Pectin determinations. Samples of approximately 500 g. of tomatoes were prepared for pectin analysis by taking portions of 10 fruits of 5 firm lines and 2 soft varieties, Waltham Forcing and Valiant, grown under field conditions. Three stages of maturity were selected to observe possible pectin changes over fairly narrow ranges of maturity which were determined by color formation. The first stage consisted of tomatoes which were colored nearly uniformly red but had some areas of green, the second of uniformly red-ripe fruit, and the third of a similar sample of red-ripe fruit allowed to stand at approximately 21°C. for 10 days after picking. The selection of the red-ripe fruits for the samples was based on two color readings taken on each whole fruit by the Hunter Color Difference Meter. The "a" and "b" components of color were plotted on graph paper and the 10 fruits with the most similar values for each line or variety were selected from a sample of 15 fruits of similar red-ripe maturity, as determined by visual observation.

The weighed samples were cut into slices of about ⅛ inch in thickness and dropped immediately into a volume of boiling 95% ethanol sufficient to make a final concentration of 35% alcohol, assuming the tomatoes contain 95% water. (It was later learned that the addition of sufficient 95% alcohol to make a final concentration of 60% alcohol would be preferable because of greater assurance of inactivating the pectin methylesterase enzyme very rapidly, and especially since that additional amount of alcohol was added later to precipitate the water-soluble pectin.) The samples were boiled for 1 minute after the last of the slices were added. The samples were then stored at 1°C. for a month before analyzing for pectin.

The samples were prepared for extraction by adding an equal volume of 95% ethanol to precipitate any pectin which may have been soluble in water in the tissue, and then by grinding the slices in a large mortar. The ground material was transferred to a nylon cloth in a large Buchner funnel and allowed to drain until nearly dry. Then 200 ml. of 95% ethanol was poured over the pulp to remove any remaining water before squeezing the pulp in the nylon cloth to remove the remaining alcohol. The alcohol-insoluble solids were transferred to large Pyrex centrifuge tubes for extraction.

Three separate fractions of pectic materials were obtained from the alcohol-insoluble solids. The water-soluble pectin was dissolved in 3 extractions with about 500 ml. of distilled water at 30°C. for 1 hour for the first, and 2 hours for the last two extracts. After each extraction the tubes were centrifuged for 15 minutes at 2000 r.p.m., after which the liquid was decanted onto a sharkskin filter paper from which any tissue could be recovered for the next fraction. The pulp was then extracted twice for 2 hours with 800 ml. of 0.5% ammonium oxalate at 30°C. to remove the low-methoxyl pectin. Finally the residue was extracted twice for 2 hours with 800 ml. of 0.05 *N.* hydrochloric acid at 85°C. to remove the remaining pectic materials including the protopectin.

The extracts were made up to volume in a 1 liter volumetric flask with distilled water and 2 ml. of toluene was added to prevent the growth of molds. The extracts were stored at 1°C. for several days before uronic acid determinations were made.

The pectin in the extracts was precipitated by a procedure similar to that employed by Carré and Haynes *(5)*. First, 400 ml. aliquots of the extracts were mixed with 150 ml. of 0.2 *N.* sodium hydroxide, and the mixture was allowed to stand for at least 1.5 hours and usually overnight to saponify. Then 60 ml. of 1 *N.* acetic acid was added and the mixture was allowed to stand for 10 minutes after which first 25 ml. of 0.1 *M.*, then 25 ml. of 2 *M.* calcium chloride solutions were added dropwise with constant, vigorous stirring to precipitate the pectin as calcium pectate. The vigorous stirring facilitates more rapid filtration by breaking up the precipitate. The mixture was then boiled for 2 minues, filtered through sharkskin filter paper, and the precipitate was washed 6 times with boiling distilled water. It was then carefully transferred to the reaction flask used in the uronic acid determinations and dried in an oven at 85°C.

The ammonium oxalate extract required an additional procedure to remove the low methoxyl pectin from the ammonium oxalate solution. This was accomplished by precipitation of the pectin with 800 ml. of 95% ethanol to a 400-ml. aliquot of the solution. The precipitate was recovered on sharkskin filter paper after centrifuging and filtering. This precipitate was washed 3 times with acidulated 60% ethanol and then dissolved in boiling water. The pectin was then precipitated as calcium pectate by the procedure described above.

The quantitative determinations of pectic materials were made by means of the uronic acid-carbon dioxide apparatus described by McCready, Swenson, and Maclay *(19)*, which measures the quantity of carbon dioxide produced upon decarboxylation of the pectic materials. The final preparation of the samples and the uronic acid determinations were made in the pectin laboratory of the New York State Agricultural Experiment Station, Geneva, New York.

A number of controls were determined to check the accuracy of the uronic acid determinations. The carbon dioxide produced by a sample of 0.2631 g. of galacturonic acid monohydrate was determined by the apparatus and found to be within 3% of the theoretical recovery of carbon dioxide upon complete decarboxylation. Two blank runs were made with the apparatus and three in titrating the sodium hydroxide. These blanks were averaged and used to correct the quantity of hydrochloric acid used in titrating the sodium hydroxide as a measure of the carbon dioxide produced. The 6 duplicate determinations of uronic acid contents made were found to differ by an average of 2.23% of the mean of each of the duplicate determinations.

Pectin methylesterase activity. The relative activity of the pectin methylesterase was determined for each of the firm lines and the two soft varieties used in comparing pectic substances and firmness. A procedure similar to the one described by Kertesz *(15)* was used for the determinations, except that brom thymol blue was used as the indicator.

RESULTS

Calcium content. Highly significant differences were indicated by the analysis of variance for calcium contents of four firm selections (49-766-3, 49-791-41, 3784-1, and 49-744), two of intermediate firmness (3781-5 and 3777-1) and the soft varieties, Valiant and Waltham Forcing, grown in well mixed soil in 12-inch pots in the greenhouse. Highly significant differences in firmness as measured by the pressure tester were also noted between these firm lines and soft varieties.

Differences in calcium content and firmness may be noted in Table 2. The firmness ratings, indicated as compression of fruit in Table 2, are 5.5 times greater than the actual distance in cms. that a 1-inch plunger compressed the fruit, because a fulcrum with an advantage of 5.5 to 1 was used to magnify compression readings to facilitate more accurate measurement of small movements of the plunger. The smallest compression readings indicate the firmest fruits.

TABLE 2

Calcium content and firmness of six firm selections and two soft varieties grown in pots in the greenhouse

Calcium content		Firmness	
Line or variety	Calcium content of tomato flesh (fresh weight)	Line or variety	Compression of fruit [b]
	%		
49-766-3 (F_4)	0.012	49-791-41	3.37
49-791-41 (F_3)	0.011	3784-1	3.98
3784-1 (F_3)	0.010	49-766-3	4.11
49-774 (F_3)	0.008	49-774	4.26
Waltham Forcing	0.008	3781-5	4.62
Valiant	0.007	3777-1	4.68
3781-5 (F_3)	0.007	Valiant	5.64
3777-1 (F_3)	0.006	Waltham Forcing	7.08
Average L.S.D.: 0.05 = 0.003		Average L.S.D.: 0.05 = 0.78	
0.01 = 0.004		0.01 = 1.03	

[b] See text for method. Small compression indicates high firmness, high compression comparatively soft tomatoes.

It is of interest to note that the only firm line with highly significantly more calcium than Valiant was an F_4, whereas all other firm lines were in the F_3 generation and showed considerable variation between plants in firmness of fruit.

Lines more nearly homozygous for firmness might be expected to show more marked differences in calcium content as compared to Valiant, as was observed by comparing a firm selection of line 49-774 (49-774-4) with Valiant grown on low, intermediate, and high levels of calcium in nutriculture. Firm selection 49-774-4 was used in this comparison because it produced fruits of superior quality to those of line 49-766-3.

The analysis of variance showed highly significant differences between varieties and nutrient levels in the contents of calcium of the fleshy tissue of fruits of the firm line 49-774-4 and Valiant grown on low, intermediate, and high levels of calcium. Highly significant differences in firmness were also indicated between varieties and nutrient levels, as determined by the analysis of variance of compression readings of the pressure tester. The difference in calcium content and firmness may be noted in Table 3.

Firming of fresh tomato tissue with calcium chloride. There was no appreciable difference in the firmness of 1-inch slices of fruits of 6 firm lines when measured without calcium chloride treatment and after soaking in calcium chloride solutions. Slices of fruits of Waltham Forcing and Valiant became much firmer after soaking in calcium chloride solutions as com-

TABLE 3

Calcium content and firmness of the firm selection 49-774-4 and Valiant grown in nutriculture on three levels of calcium

Nutrient level of calcium	Calcium content (dry weight basis)		Firmness (compression of fruits)[c]	
	49-774-4	Valiant	49-774-4	Valiant
	%	%	%	%
High	0.349	0.289	1.26	2.78
Medium	0.290	0.191	1.94	2.89
Low	0.132	0.081	2.53	3.24

L.S.D.:
Between nutrient levels: $0.05 = 0.080$, $0.01 = 0.113$
Between varieties: $0.05 = 0.072$, $0.01 = 0.096$ [d]

L.S.D. between nutrient levels and varieties:
$0.05 = 0.44$
$0.01 = 0.58$

[c] See footnote [b], Table 2.

[d] The r between calcium content and compression of fruit is -0.783.

pared to similar untreated slices used as a check. This rather striking difference is shown in Figure 1.

Pectin determinations. Marked differences were noted between the firm selection 50-338-5 (which is a further selection from 49-774-4) and Valiant in the water-soluble, ammonium oxalate-soluble, and acid-soluble pectic fractions and in total contents of pectin. These differences were consistent for three stages of maturity as indicated in Table 4. There is little

TABLE 4

Three fractions and total pectic materials of fruits of Valiant and the firm selection 50-338-5 for three stages of maturity (expressed as polygalacturonic acid on fresh weight basis)

	Variety	Underripe	Ripe	Overripe
Water-soluble pectin, %	Valiant	0.043	0.032	0.039
	50-338-5	0.073	0.066	0.041
Ammonium oxalate-soluble pectin, %	Valiant	0.041	0.047	0.056
	50-338-5	0.080	0.071	0.091
Acid-soluble pectin, %	Valiant	0.050	0.046	0.053
	50-338-5	0.103	0.108	0.093
Total pectin, %	Valiant	0.134	0.124	0.148
	50-338-5	0.256	0.246	0.225

difference in the pectin content of Valiant or line 50-338-5 over the range of maturity observed.

Since selection 50-338-5 was found to contain approximately twice as much pectin as Valiant, additional firm lines and the variety Waltham Forcing (which produces relatively soft fruits but the firmest of the commercial varieties tested) were compared in the red-ripe stage of maturity to determine whether the differences in pectin were consistent with the firmness of the fruits. The same three pectic fractions were determined. All of the firm selections contained considerably more of the three fractions and total pectin than Valiant and Waltham Forcing and the differences in the

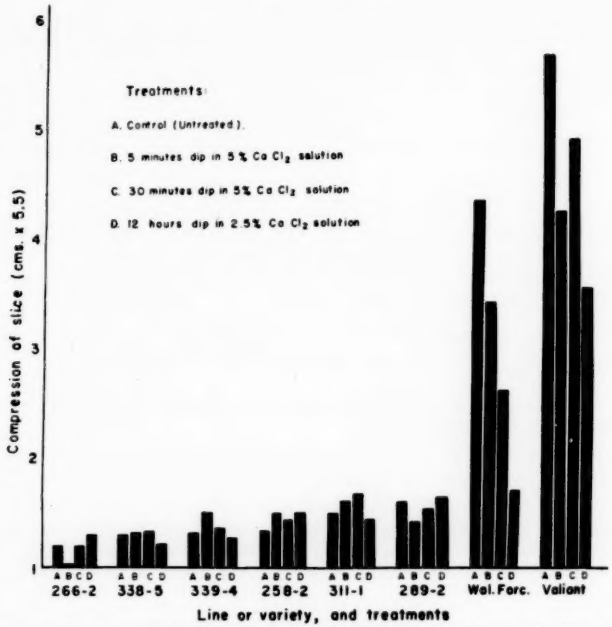

Figure 1. The relative firming effect of 3 treatments of calcium chloride on 1-inch slices of fruits of 6 firm lines and Waltham Forcing and Valiant. Compression readings are averages for 5 1-inch slices.

pectic content were consistent with differences in firmness (Table 5) in that all five firm lines which do not differ significantly in firmness contain considerably more pectin than Waltham Forcing or Valiant.

Correlation coefficients were computed between the three fractions and total pectin and an average compression reading determined by the pressure

TABLE 5

Three fractions and the total content of pectic substances in red-ripe fruits of five firm lines and Waltham Forcing and Valiant (expressed as per cent polygalacturonic acid on fresh weight)

Line or variety (Ranked according to firmness)	Content of pectic substances			
	Water-soluble	Ammonium oxalate-soluble	Acid-soluble	Total
	%	%	%	%
50-338-5	0.066	0.071	0.108	0.246
50-332-4	0.064	0.103	0.133	0.300
50-339-4	0.057	0.074	0.078	0.209
50-258-2	0.072	0.071	0.115	0.258
50-311-1	0.064	0.083	0.090	0.238
Waltham Forcing	0.052	0.051	0.055	0.158
Valiant	0.032	0.047	0.046	0.124

tester. The contents of water-soluble, ammonium oxalate-soluble, and acid-soluble pectin were correlated with compression readings at odds of 19 to 1 with coefficients of —0.83, —0.78, and —0.86, respectively. Total pectin and compression readings are correlated at odds of 99 to 1 with an "r" value of —0.88.

Pectin methylesterase activity. Highly significant differences between the relative activity of the enzyme pectin methylesterase for each of the firm lines and the two soft varieties used in comparing pectic substances and firmness were indicated by the analysis of variance and are shown in Table 6. The values indicating relative enzyme activity correspond to the amounts of 0.1 *N*. sodium hydroxide required to neutralize the free carboxyl groups produced by 1 ml. of enzyme extract in 50 ml. of 1% citrus pectin in 10 minutes. The values are indicated as relative since the enzyme activity was measured at a pH of 7.05 and therefore the results are not directly comparable to most activities reported in the literature.

TABLE 6

Relative pectin methylesterase activity of five firm lines and Waltham Forcing and Valiant (expressed as ml. of 0.1 N. sodium hydroxide used in titrating)

Line or variety	Relative pectinmethylesterase activity
50-258-2	4.81
50-311-1	4.65
50-332-4	4.01
50-338-5	2.85
50-339-4	2.37
Waltham Forcing	1.80
Valiant	1.26

DISCUSSION

The differences in the calcium content between tissues of firm and soft tomatoes do not appear to be great enough to fully account for the differences in firmness noted between the firm lines and soft varieties of tomatoes used in this study even though the firming effect of additions of calcium was quite apparent when slices of fruits of the varieties Waltham Forcing and Valiant were immersed in calcium chloride solutions. The very marked differences in water-soluble, ammonium oxalate-soluble, acid-soluble, and total pectic materials certainly appear to be associated with differences in firmness. A possible influence of the enzyme pectin methylesterase on firmness of tomatoes is suggested by the consistently greater activity of the enzyme in firm fruits.

The firmness of the tomato fruits produced by the firm lines used in this study appears to be conditioned in part by larger contents of calcium which forms the tissue-firming calcium pectate gel with low methoxyl pectin. A higher content of low methoxyl pectin and a greater activity of pectin methylesterase result in an increased supply of low methoxyl pectin to form the calcium pectate gel with the increased calcium content.

The increased amount of acid-soluble pectin or protopectin also may add materially to the firmness of the tissue by cementing adjacent cells together and by thus adding to the rigidity and firmness of the tissues.

SUMMARY

Calcium contents were determined for flesh of fruits of four firm lines, two of intermediate firmness, and the relatively soft fruited varieties Valiant and Waltham Forcing which were grown in the greenhouse on well mixed soil of high calcium content. Several of the firm lines contained more calcium in the fleshy portions of the fruits than Valiant or Waltham Forcing.

A firm line accumulated more calcium in the fleshy portion of the fruits than Valiant when grown in nutriculture on low, intermediate, and high levels of calcium and boron. As the calcium supply was increased the calcium content of the fruits of both the firm line and Valiant increased and they were firmer. The firm line showed a greater increase in firmness when grown on high calcium than did Valiant.

One-inch slices from fruits of Valiant and Waltham Forcing became considerably firmer when immersed in solutions of calcium chloride, whereas no appreciable increase in firmness was noted for six firm lines.

Five firm selections were found to contain considerably more of the pectic materials than Valiant and Waltham Forcing. This relationship was observed for contents of water-soluble pectin, ammonium oxalate-soluble or low methoxyl pectin, acid-soluble or protopectin, and total pectic materials. The content of each pectic fraction and total pectin is correlated with firmness at odds of 19 to 1 for the three fractions, and at odds of 99 to 1 for total pectin.

Fruits of five firm lines exhibit considerably greater activity of the enzyme pectin methylesterase than do fruits of Valiant and Waltham Forcing.

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LITERATURE CITED

1. Allen, C. E. On the origin and nature of the middle lamella. *Botan. Gaz.*, **32**, 1 (1901).
2. Appleman, C. O., and Conrad, C. M. The pectic constituents of tomatoes and their relation to the canned product. Maryland Agr. Expt. Sta., *Bull.*, **291** (1927).
3. Beeson, K. C. Ionic absorption by tomato plants as correlated with variations in the composition of the nutrient medium. *Plant Physiol.*, **19**, 258 (1944).
4. Carré, M. H. Investigations on the pectic constituents of apples. *Ann. Botany*, **39**, 811 (1925).
5. Carré, M. H., and Haynes, D. The estimation of pectin as calcium pectate and the application of this method to the determination of the soluble pectin in apples. *Biochem. J.*, **16**, 60 (1922).
6. Emmett, A. M. An investigation on the changes which take place in the chemical composition of pears stored at different temperatures, with special reference to the pectic changes. *Ann. Botany*, **43**, 269 (1929).
7. Gaddum, L. W. The pectic constituents of citrus fruits. Florida Agr. Expt. Sta., *Bull.*, **268** (1934).
8. Haller, M. H. Changes in the pectic constituents of apples in relation to softening. *J. Agr. Research*, **39**, 739 (1929).

9. HAMSON, A. R. A pressure tester developed to measure firmness in tomatoes. *Proc. Am. Soc. Hort. Sci.* (In press.)

10. HARVEY, P. H. Hereditary variations in plant nutrition. *Genetics*, **24**, 437 (1939).

11. HOAGLAND, D. R., AND ARNON, D. I. The water-culture method for growing plants without soil. Calif. Agr. Expt. Sta., *Circ.*, **347** (1938).

12. HOPKINS, E. F., AND GOURLEY, J. H. Nitrogen fertilization and pectic materials in grapes. *Proc. Am. Soc. Hort. Sci.*, **27**, 164 (1930).

13. KASSAB, M. A. Observations on the pectin content of California tomatoes. *Fruit Products J.*, **11**, 69 (1931).

14. KERTESZ, Z. I. The effect of calcium on plant tissues. *Canner*, **88** (7) 26 (1939).

15. KERTESZ, Z. I. *The Pectic Substances*. 1951, Interscience Publishers, Inc. New York, N. Y.

16. KERTESZ, Z. I., AND LOCONTI, J. D. Factors determining the consistency of commercial canned tomato juice. N. Y. State Agr. Expt. Sta., *Tech. Bull.*, **272** (1944).

17. LECRONE, F., AND HABER, E. S. Changes in the pectic constituents of tomatoes in storage. *Iowa State Coll. J. Sci.*, **7**, 467 (1933).

18. LOCONTI, J. D., AND KERTESZ, Z. I. Identification of calcium pectate as the tissue firming compound formed by treatment of tomatoes with calcium chloride. *Food Research*, **6**, 499 (1941).

19. MCCREADY, R. M., SWENSON, H. A., AND MACLAY, W. D. Determination of uronic acids. *Ind. Eng. Chem., Anal. Ed.*, **18**, 290 (1946).

20. REITEMEIER, R. F. The semi-micro analysis of soil solutions of saline soils. Mimeograph U. S. Regional Salinity Laboratory, Bur. Plant Industry, Riverside, California.

21. SAYRE, C. B., KERTESZ, Z. I., AND LOCONTI, J. D. Effect of calcium and potassium fertilizers on the solidity and calcium and potassium content of canned tomatoes. *J. Am. Soc. Agron.*, **32**, 389 (1940).

22. SAYWELL, L. G., AND CRUESS, W. V. Composition of canning tomatoes. Calif. Agr. Expt. Sta., *Bull.*, **545** (1932).

23 SMITH, S. N. Response of inbred lines and crosses in maize to variations of nitrogen and phosphorous supplied as nutrients. *J. Am. Soc. Agron.*, **26**, 785 (1934).

24. WEISS, M. G. Inheritance and physiology of efficiency in iron utilization in soybeans. *Genetics*, **28**, 253 (1943).

HEAT PROCESSING OF BEEF

I. A THEORETICAL CONSIDERATION OF THE DISTRIBUTION OF TEMPERATURE WITH TIME AND IN SPACE DURING PROCESSING[a, b]

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The classical work of Fourier (*6*) in developing the theory of heat conduction is the basis of the more recent work of Carslaw (*3*) and Carslaw and Jaeger (*4*). This theory has been applied to problems in the food industry by Thompson (*13*), Ball (*1, 2*), Jackson and Olson (*9*), Olson and Schultz (*11*), and Merrill (*10*). None of these efforts was concerned with the canning of beef and the derivations of formulas given were incomplete.

The present work was undertaken to derive an expression based on the fundamental concepts of heat conduction which would be useful in mapping the course of temperature changes in a cylindrical container of beef in terms of series of isothermal surfaces.

Thompson (*13*) used a mathematical relationship developed from Carslaw (*3*) for the flow of heat in unsteady state into a finite cylinder. This expression may be stated in the following form:

$$v = C - v_o \sum_{\mu=1}^{\infty} \sum_{m=1}^{\infty} A_{\mu m} e^{-k(\mu^2+\lambda^2)t} J_o(\mu r) \sin \lambda (z + l)$$

where: v is the temperature within the cylinder, t is time, C the temperature of the bath, v_o the initial temperature difference, r,z the cylindrical coordinates of space, μa the zero of $J_o(\mu a)$, a the radius of the cylinder, $2l$ the height of the cylinder, k the thermal diffusivity, λ equals $\frac{m\pi}{2l}$, and $A_{\mu m}$ equals $\frac{4(1 - \cos m\pi)}{m\pi(\mu a) J_1(\mu a)}$, a constant depending on the initial temperature distribution in the cylinder. This equation is a solution of a boundary value problem encountered in this investigation. This problem has been restated briefly by Hicks (*7*).

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[b] This paper reports research sponsored by the Quartermaster Food and Container Institute for the Armed Forces, and has been assigned number 367 in the series of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the Department of Defense.

DERIVATION OF THE HEAT CONDUCTION EQUATION

The general equation of heat conduction may be derived for any set of conditions and its derivation may be found in Fourier (*6*), Carslaw and Jaeger (*4*), and in particular in Ingersoll and Zobel (*8*). Consider a rectangular parallelepiped in Cartesian coordinates (Figure 1) with sides of

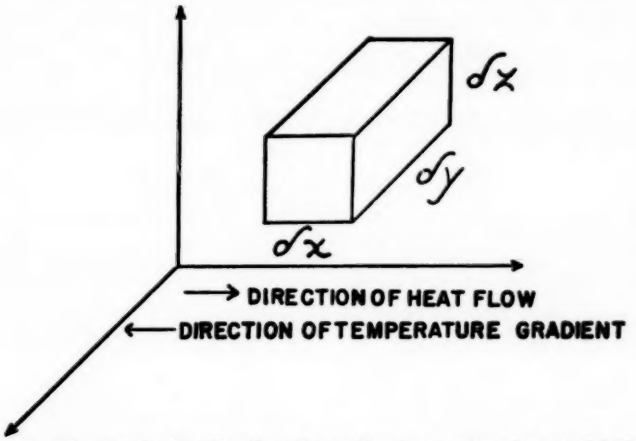

Figure 1. Diagram showing direction of heat flow in a rectangular parallelepiped.

length δx, δy, δz. Assume that the temperature at the center of the parallelepiped $\delta x \delta y \delta z$ is equal to v. Since this volume is very small, it is assumed that the temperature at each surface is uniform and different from the temperature at the center.

If $\frac{\partial v}{\partial x}$ equals the temperature gradient in the x direction (change of temperature per unit length) then the temperature at the left surface is

$$v - \frac{1}{2}\frac{\partial v}{\partial x}\delta x \tag{1}$$

and at the right surface

$$v + \frac{1}{2}\frac{\partial v}{\partial x}\delta x. \tag{2}$$

The basic equation of heat conduction may be stated as:

$$\frac{\partial Q}{\partial t} = -KA\frac{\partial v}{\partial x}, \tag{3}$$

where Q is the quantity of heat in B.t.u.; t is the time; K is the conductivity, B.t.u. per (hr.)(ft.)(°F.); v is the temperature; and A is the area. Substituting (1) into (3) we obtain

$$\frac{\partial Q}{\partial t} = -K\delta y\delta z\frac{\partial}{\partial x}\left[v - \frac{1}{2}\frac{\partial v}{\partial x}\delta x\right] \tag{4}$$

for the flow of heat through the left surface in unit time. Similarly, substituting (2) into (3) we obtain

$$\frac{\partial Q}{\partial t} = -K\delta y\delta z\frac{\partial}{\partial x}\left[v + \frac{1}{2}\frac{\partial v}{\partial x}\delta x\right] \tag{5}$$

for the flow of heat through the right surface.

The net gain in heat for the volume being considered is for the flow in the x direction obtained by subtracting Equation 5 from Equation 4.

$$-K\delta y\delta z\frac{\partial}{\partial x}\left(v - \frac{1}{2}\frac{\partial v}{\partial x}\delta x\right) + K\delta y\delta z\frac{\partial}{\partial x}\left(v + \frac{1}{2}\frac{\partial v}{\partial x}\delta x\right) = Q_{\text{net}}$$

$$K\delta y\delta z\left[-\frac{\partial}{\partial x}\left(v - \frac{1}{2}\frac{\partial v}{\partial x}\delta x\right) + \frac{\partial}{\partial x}\left(v + \frac{1}{2}\frac{\partial v}{\partial x}\delta x\right)\right] = Q_{\text{net}}.$$

Differentiating and keeping in mind that δx is a constant, it is found that

$$K\delta y\delta z\left[-\frac{\partial v}{\partial x} + \frac{1}{2}\frac{\partial^2 v}{\partial x^2}\delta x + \frac{\partial v}{\partial x} + \frac{1}{2}\frac{\partial^2 v}{\partial x^2}\delta x\right] = Q_{\text{net}},$$

and by simplifying, the difference in heat flow between the $\delta y\delta z$ faces is:

$$K\delta y\delta z\delta x\frac{\partial^2 v}{\partial x^2}, \tag{6}$$

and by analogy,

$$K\delta x\delta z\delta y\frac{\partial^2 v}{\partial y^2} \text{ and} \tag{7}$$

$$K\delta x\delta y\delta z\frac{\partial^2 v}{\partial z^2} \tag{8}$$

are the differences in heat flow in y and z direction, respectively. Therefore, the total quantity of heat gained by the $\delta x\delta y\delta z$ element in unit time is obtained by addition of (6), (7), and (8) as:

$$K\delta x\delta y\delta z\left(\frac{\partial^2 v}{\partial x^2} + \frac{\partial^2 v}{\partial y^2} + \frac{\partial^2 v}{\partial z^2}\right). \tag{9}$$

If c_p = the specific heat at constant pressure, and ρ = the density of the substance being heated then the total heat gained in unit time is

$$c_p\rho\delta x\delta y\delta z\frac{\partial v}{\partial t}. \tag{10}$$

Since the total heat gained is expressed either as Equation 9 or Equation 10, they must be equal:

$$K\left(\frac{\partial^2 v}{\partial x^2}+\frac{\partial^2 v}{\partial y^2}+\frac{\partial^2 v}{\partial z^2}\right)=c_p\rho\frac{\partial v}{\partial t}. \tag{11}$$

If the thermal diffusivity of the substance being heated is defined as $k=\dfrac{K}{c_p\rho}$ and is found substantially constant then

$$\frac{\partial v}{\partial t}=k\left(\frac{\partial^2 v}{\partial x^2}+\frac{\partial^2 v}{\partial y^2}+\frac{\partial^2 v}{\partial z^2}\right) \tag{12}$$

and substituting the Laplacian operator ∇^2, Equation 12 becomes

$$\frac{\partial v}{\partial t}=k\nabla^2 v. \tag{13}$$

This is Fourier's equation for the flow of heat in an isotropic body, where the diffusivity is assumed constant and the same in all directions of flow of heat. All heat conduction problems must be satisfied by this equation. Since the case at hand involves cylindrical symmetry, it is desirable to express Equation 13 in cylindrical coordinates. In cylindrical coordinates and for the case of two-dimensional flow (in the r direction and z direction)

$$r^2=x^2+y^2, \text{ and}$$

$$\frac{\partial r}{\partial x}=\frac{x}{r},\quad \frac{\partial r}{\partial y}=\frac{y}{r}\quad\text{and}\quad \frac{\partial v}{\partial x}=\frac{\partial v}{\partial r}\cdot\frac{\partial r}{\partial x}=\frac{\partial v}{\partial r}\cdot\frac{x}{r}$$

$$\frac{\partial^2 v}{\partial x^2}=\frac{\partial}{\partial x}\left[\frac{\partial v}{\partial r}\cdot\frac{x}{r}\right]=\frac{\partial}{\partial r}\left[\frac{\partial v}{\partial r}\cdot\frac{x}{r}\right]\frac{\partial r}{\partial x}$$

$$=\frac{\partial r}{\partial x}\left[\frac{\partial v}{\partial r}\frac{\partial}{\partial r}\left(\frac{x}{r}\right)+\frac{x}{r}\frac{\partial^2 v}{\partial r^2}\right]$$

$$=\frac{\partial r}{\partial x}\left[\frac{\partial v}{\partial r}\left(\frac{r\dfrac{\partial x}{\partial r}-x}{r^2}\right)+\frac{x}{r}\frac{\partial^2 y}{\partial r^2}\right]$$

$$=\frac{x}{r}\left[\frac{\partial v}{\partial r}\left(\frac{1}{r}\frac{\partial x}{\partial r}-\frac{x}{r^2}\right)+\frac{x}{r}\frac{\partial^2 v}{\partial r^2}\right]$$

$$=\frac{x}{r}\left[\frac{\partial v}{\partial r}\frac{1}{r}\frac{r}{x}-\frac{\partial v}{\partial r}\cdot\frac{x}{r^2}+\frac{x}{r}\frac{\partial^2 v}{\partial r^2}\right]$$

$$=\frac{\partial v}{\partial r}\frac{1}{r}-\frac{\partial v}{\partial r}\frac{x^2}{r^3}+\frac{\partial^2 v}{\partial r^2}\frac{x^2}{r^2}$$

and therefore

$$\frac{\partial^2 v}{\partial x^2}=\frac{x^2}{r^2}\frac{\partial^2 v}{\partial r^2}+\frac{1}{r}\frac{\partial v}{\partial r}-\frac{x^2}{r^3}\frac{\partial v}{\partial r}. \tag{14.1}$$

Similarly

$$\frac{\partial^2 v}{\partial y^2}=\frac{y^2}{r^2}\frac{\partial^2 v}{\partial r^2}+\frac{1}{r}\frac{\partial v}{\partial r}-\frac{y^2}{r^3}\frac{\partial v}{\partial r}, \tag{14.2}$$

whereas $\dfrac{\partial^2 v}{\partial z^2}$ remains unchanged in cylindrical coordinates. (14.3)

Adding both sides of 14.1, 14.2 and 14.3 we obtain

$$\nabla^2 v = \left(\frac{\partial^2 v}{\partial x^2} + \frac{\partial^2 v}{\partial y^2} + \frac{\partial^2 v}{\partial z^2}\right) = \left(\frac{x^2+y^2}{r^2} \cdot \frac{\partial^2 v}{\partial r^2} + \frac{2}{r}\frac{\partial v}{\partial r} - \frac{x^2+y^2}{r^3} \cdot \frac{\partial v}{\partial r} + \frac{\partial^2 v}{\partial z^2}\right),$$

which upon simplifying gives

$$\nabla^2 v = \left(\frac{\partial^2 v}{\partial r^2} + \frac{1}{r}\frac{\partial v}{\partial r} + \frac{\partial^2 v}{\partial z^2}\right). \tag{14}$$

Combining with Equation 13 we obtain the Fourier equation in cylindrical coordinates as

$$\frac{\partial v}{\partial t} = k\nabla^2 v = k\left(\frac{\partial^2 v}{\partial r^2} + \frac{1}{r}\frac{\partial v}{\partial r} + \frac{\partial^2 v}{\partial z^2}\right). \tag{14a}$$

Equation 14a is the general equation for two dimensional flow (radial, r direction; axial, z direction) for the case of a finite cylinder. In this discussion no attention will be given to the solution of equations for cylinders with other shapes of cross sections, or for the slab or sphere, etc., which may be derived by following lines of reasoning similar to those used for a finite cylinder.

Statement of Boundary and Initial Conditions

The circular cylindrical shape being considered suggests the use of Bessel functions (cylindrical functions) in conjunction with exponential and trigonometric functions in the solution of the differential equation 14a restated here as

$$\text{I} \qquad \frac{\partial v}{\partial t} = k\left(\frac{\partial^2 v}{\partial r^2} + \frac{1}{r}\frac{\partial v}{\partial r} + \frac{\partial^2 v}{\partial z^2}\right) = k\nabla^2 v.$$

In order to obtain an exact solution of a differential equation, the initial and boundary conditions must be specified. For the cylindrical cross-section shown in Figure 2 and when k (thermal diffusivity) is considered constant and v is a temperature function, the boundary conditions are as follows:

$$v(r,z,t)\Big|_{r=a} = v(r,z,t)\Big|_{z=0} = v(r,z,t)\Big|_{z=2l} = \begin{cases} RT \text{ for } 0 < t < t_1 \\ TC \text{ for } t > t_1 \end{cases} \tag{15}$$

where RT is retort temperature, TC is cooling temperature, and t_1 is time when heating is terminated; whereas initial conditions are:

$$v(r,z,t)\Big|_{t=0} = IT \tag{16}$$

where IT is initial temperature.

Conditions shown in Equation 15 mean that at any time $t > 0$ of processing the surface of the cylinder is at retort temperature for the heating process and at cooling water temperature for the cooling process. Condition 16 indicates uniform temperature distribution throughout the isotropic body including the boundaries at the beginning of the process.

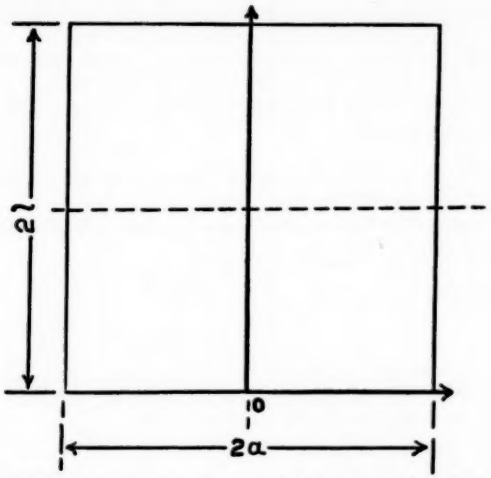

Figure 2. Vertical cross section of a cylindrical can showing the location of the axes with the origin at 0.

Transformation of Temperature Function $v(r,z,t)$.

II As a first step toward the solution let $v - IT = u$, to affect ultimately the homogeneity of the boundary conditions. Then $\frac{\partial u}{\partial t} = k\nabla^2 u$ and the boundary conditions (at the surface of the container) become:

$$u(r,z,t)\Big|_{r=a} = u(r,z,t)\Big|_{z=0} = u(r,z,t)\Big|_{z=2l} = \begin{cases} RT - IT \text{ for } 0 < t < t_1 \\ TC - IT \text{ for } t > t_1 \end{cases} \quad (17)$$

Since $v = RT$ at the boundary for heating and $v = TC$ for cooling, initial conditions become

$$u(r,z,t)\Big|_{t=0} = 0 .$$

III In similar fashion making a new transformation for the heating period, $(t < t_1)$, let $\frac{u_t}{RT - IT} = \frac{v - IT}{RT - IT} = W_t$ or $u_t = W_t(RT - IT)$ then the boundary conditions for heating are:

$$W_t(a,z,t) = W_t(r,0,t) = W_t(r,2l,t) = \begin{cases} \frac{v - IT}{RT - IT} = \frac{RT - IT}{RT - IT} = 1 \text{ for } 0 < t < t_1 \\ 0 \qquad\qquad\qquad\qquad \text{for } t > t_1 \end{cases} \quad (18a)$$

for any time during the heating period as $v = RT$ at the surface, and $IT_{\text{cooling}} = RT_{\text{heating}}$.

Now to obtain an expression of the same form for the cooling period ($t > t_1$) starting at t_1 let $\frac{u_{t_1}}{TC - IT} = \frac{v - IT_{t_1}}{TC - IT_{t_1}} = W_{t_1}$ but at t_1 the initial temperature at the boundary $= RT$ or $IT_{\text{cooling}} = RT_{\text{heating}}$ and retort temperature is equal to TC:

$$\frac{u_{t_1}}{TC - RT} = \frac{v - RT}{TC - RT} = W_{t_1} \text{ or } u_{t_1} = W_{t_1}(TC - RT)$$

which gives similar boundary conditions for the cooling period to those of (18a) for the heating period: (18b)

$$W_{t_1}(a,z,t_1) = W_{t_1}(r,0,t_1) = W_{t_1}(r,2l,t_1) = \begin{cases} 0 & \text{for } 0 < t < t_1 \\ \dfrac{v - RT}{TC - RT} = \dfrac{TC - RT}{TC - RT} = 1 & \text{for } t > t_1 \end{cases}$$

because v at the boundary is equal to TC for cooling and to RT for heating. Then $W = W_t + W_{t_1}$ will be the solution for both the heating and cooling period (total process). The initial conditions are:

a) for the heating period ($t < t_1$)

$$W_t = W(r,z,t)\Big|_{t=0} \quad \text{because} \quad \begin{cases} v_{t=0} = IT \text{ and} \\ W_t = \dfrac{v - IT}{RT - IT} = 0 \end{cases}$$

$$= W(r,z,0) \quad = 0$$

b) for the cooling period ($t > t_1$)

$$W_{t_1} = W(r,z,t)\Big|_{t=t_1} \quad \text{because} \quad \begin{cases} v_{t=t_1} = RT \text{ and} \\ W_{t_1} = \dfrac{v - RT}{RT - IT} = 0 \end{cases}$$

$$= W(r,z,t_1) \quad = 0$$

changing the time reference to $(t - t_1)$ where t_1 = time when the cooling period is started.

$$W_{t_1}(r,z,t - t_1) = W(r,z,0)_{(\text{for } t = t_1)} = 0$$

and then in general

$$\frac{\partial W}{\partial t} = k\nabla^2 W \tag{19a}$$

with boundary conditions

$$W(a,z,t) = W(r,0,t) = W(r,2l,t) = 1 \tag{19b}$$

and initial conditions

$$W(r,z,0) = 0. \tag{19c}$$

The solution for W is composed of two parts:

(a) $W(r,z,t)$ for $0 < t < t_1$
(b) $W(r,z,t - t_1)$ for $t > t_1$ so that:
$W = W(r,z,t) + W(r,z,t - t_1)$

and to satisfy II

$$u = u_t + u_{t_1}$$
$$u = W_t(RT - IT) + W_{t_1}(TC - RT) \tag{20a}$$

which at the boundary has for all the times $W_t = W_{t_1} = 1$, and $u = RT - IT + TC - RT = (TC - IT)$ for the cooling period, and $u = W_t(RT - IT) = (RT - IT)$ for the heating period [because $TC - RT = 0$ during cooling (cooling temperature = retort temperature)]. Also

$$u = v(r,z,t) - IT \text{ (from II).} \tag{20b}$$

Now combining (20a) and (20b),

$$v(r,z,t) = IT + (RT - IT)\, W(r,z,t) + \begin{cases} 0 & \text{for } 0 < t < t_1 \\ (TC - RT)\, W(r,z,t-t_1) & \text{for } t > t_1 \end{cases} \tag{20c}$$

and where

$$W_t = W(r,z,t) \text{ and } W_{t_1} = W(r,z,t - t_1).$$

The problem is then reduced to solving III. To simplify and to obtain homogeneous boundary conditions (the value of the function at the boundary = 0), let

$$W - 1 = S \text{ and since } \frac{\partial(-1)}{\partial t} = 0$$

the problem is as follows:

$$\text{IV} \quad \begin{cases} \dfrac{\partial S}{\partial t} = k\left(\dfrac{\partial^2 s}{\partial r^2} + \dfrac{1}{r}\dfrac{\partial S}{\partial r} + \dfrac{\partial^2 S}{\partial z^2}\right) = k\nabla^2 S & (21) \\ S(a,z,t) = S(r,0,t) = S(r,2l,t) = 0 \quad \text{(boundary conditions)} & (22) \\ S(r,z,0) = -1 \quad \text{(initial conditions).} & (23) \end{cases}$$

Solution of the Heat Equation

To separate variables let

$$S = R(r)\, Z(z)\, T(t) \tag{24}$$

where $R(r)$ is a function of r only, $Z(z)$ is a function of z only, and $T(t)$ is a function of t only. Substituting (24) in (21) and differentiating $\left(\text{as for instance } \dfrac{\partial S}{\partial t} = \dfrac{\partial (RZT)}{\partial t} = RZ \cdot \dfrac{dT}{dt} = RZT'\right)$ we obtain;

$$k\left(R''ZT + \frac{1}{r}R'ZT + RZ''T\right)' = RZT'$$

dividing by kT and RZ;

$$\frac{R''}{R} + \frac{\frac{1}{r}R'}{R} + \frac{Z''}{Z} = \frac{T'}{kT}$$

yielding;

$$\frac{R'' + \frac{1}{r}R'}{R} + \frac{Z''}{Z} = \frac{T'}{kT}.$$

Neither of the members of this expression can change with a change in the other and, therefore, each separately is a constant.

Let then

$$\frac{R'' + \frac{1}{r} R'}{R} = -\mu^2$$

which gives

$$R'' + \frac{1}{r} R' + \mu^2 R = 0, \tag{25}$$

and let

$$\frac{Z''}{Z} = -\lambda^2$$

which gives

$$Z'' + \lambda^2 Z = 0$$

and then

$$\frac{T'}{kT} = -(\mu^2 + \lambda^2)$$

which gives

$$T' - k(\mu^2 + \lambda^2) T = 0. \tag{27}$$

To obtain the solution of (21), now divided into 3 separate equations, it is necessary to solve for μ and λ. The boundary conditions contained in (22) will provide means for it, and so:
Equation 25 has to satisfy the boundary condition derived from

$$S(a,z,t) = 0$$

$$R(r)\Big|_{r=a} = R(a) = 0. \tag{25a}$$

Equation 26 has to satisfy the boundary conditions derived from

$$S(r,0,t) = S(r,2l,t) = 0$$

$$Z(z)\Big|_{z=0} = Z(z)\Big|_{z=2l} = 0 \text{ or}$$

$$Z(0) = Z(2l) = 0. \tag{26a}$$

Now Equation 25 is a Bessel equation of zero order.
The general form of a Bessel equation is

$$x^2 \frac{d^2R(x)}{dx^2} + \frac{dR(x)}{dx} + (x^2 - n^2) R(x) = 0 \tag{25c}$$

and any solution of this equation is called a Bessel function which has a particular solution in the form of a power series (*5*, p. 143) . . . $J_n(x)$ for $(n = 1, 2 \ldots)$; for $n = 0$ the function of order zero is obtained.

$$R(x) = J_0(x) = 1 - \frac{x^2}{2^2} + \frac{x^4}{2^2.4^2} - \frac{x^6}{2^2.4^2.6^2} + \ldots$$

which is the solution of Equation 25c with $n = 0$.

$$x^2 \frac{d^2R}{dx^2} + x \frac{dR}{dx} + x^2 R = 0 \text{ or}$$

$$x^2 R'' + xR' + x^2 R = 0 \text{ or}$$

$$x^2 J''(x) + J'(x) + x^2 J(x) = 0.$$

Substituting a new variable r where $x = \mu r$ (μ a constant)

$$(\mu r)^2 \frac{d^2R}{\mu^2 dr^2} + (\mu r)\frac{dR}{\mu dr} + \mu^2 r^2 R = 0$$

is obtained, simplifying and dividing by r^2

$$\frac{d^2R}{dr^2} + \frac{1}{r}\frac{dR}{dr} + \mu^2 R = 0 \tag{28}$$

where

$$R(x) = R(\mu r) = J_o(\mu r).$$

Equation 28 is identical with Equation 25, and therefore

$$R(r) = J_o(\mu_i r) \tag{25b}$$

with the boundary condition (25a) transformed into $J_o(\mu_i a) = 0$ where μ_i is a root of $J_o(\mu_i a) = 0$ and where $\mu_i a = x_i$ are zeros of the $J_o(x)$ and which are infinite in number (*5*, p. 158) ($i = 1, 2 \ldots$). Thus the value of μ_i has been determined functionally.

The general solution of Equation 26 with the boundary conditions (26a) which is a linear homogeneous ordinary differential equation with constant coefficients is (*5*, p. 26):

$Z = A \sin \lambda z + B \cos \lambda z$ with A and B arbitrary constants. To satisfy the condition $Z(0) = 0$, B must vanish and $A \neq 0$, not to obtain a trivial solution $Z(z) = 0$. To satisfy $Z(2l) = 0$, $\sin \lambda 2l = 0$ which will be the case if

$$\lambda_m = \frac{m\pi}{2l} \quad (m = 1, 2 \ldots)$$

and the solution is

$$Z = A \sin \frac{m\pi}{2l}(z).$$

Shifting the origin of the coordinates to the center of the container

$$Z = A \sin \frac{m\pi\,(z + l)}{2l} \tag{26b}$$

with the boundary conditions

$$Z(-l) = Z(l) = 0.$$

Thus the values of μ_i and λ_m have been determined functionally. The solution of Equation 27 is (*12*, p. 108)

$$T = Ce^{-k(\mu_i^2 + \lambda_m^2)t} = Ce^{-k\left(\mu_i^2 + \frac{m^2\pi^2}{4l^2}\right)t}. \tag{27a}$$

The general solution then for $S_{im} = R_i Z_m T_{im} = S_{11} + S_{12} + S_{13} + \ldots$ will be the linear combination of the RZT products in the above series of solutions [(25b), (26c), (27a)]

$$S(r,z,t) = \sum_{i=1}^{\infty}\sum_{m=1}^{\infty} N_{im} J_o(\mu_i r) \sin \frac{m\pi}{2l}(z + l)\, e^{-k(\mu_i^2 + \lambda_m^2)t}. \tag{29}$$

To satisfy the initial condition of $S(r,z,t) = -1$ for $t = 0$, Equation 29 must be equal to -1. This condition will be satisfied if the N_{im} are coefficients in the expansion into a Fourier-Bessel series of (29) with $t = 0$ or of

$$-1 = \sum_{i=1}^{\infty}\sum_{m=1}^{\infty} N_{im} J_o(\mu_i r) \sin \frac{m\pi}{2l}(z + l). \tag{29a}$$

Thus we have to evaluate the Fourier-Bessel coefficients from the above relationship. Set

$$-1 = \sum_{m=1}^{\infty}\left[\sum_{i=1}^{\infty} N_{im}J_o(\mu_i r)\right]\sin\frac{m\pi}{2l}(z+l)$$

$$-1 = \sum_{m=1}^{\infty} N_m \sin\frac{m\pi}{2l}(z+l)$$

and obtain N_m from a Fourier expansion as

$$N_m = \frac{1}{l}\int_{-l}^{l}(-1)\sin\frac{m\pi}{2l}(z+l)\,dz = \sum_{i=1}^{\infty} N_{im}J_o(\mu_i r)$$

and obtain N_{im} from a Bessel expansion as

$$N_{im} = \frac{2}{a^2[J_1(\mu_i a)]^2}\int_0^a rN_m J_o(\mu_i r)\,dr$$

[See Churchill (*5*) p. 171] and

$$N_{im} = \frac{2\int_0^a rJ_o(\mu_i r)\,dr\int_{-l}^{l}(-1)\sin\frac{m\pi}{2l}(z+l)\,dz}{la^2\,[J_1(\mu_i a)]^2}.$$

Integrating between limits

$$N_{im} = \frac{2a\,J_1(\mu_i a)\int_{-l}^{l}(-1)\sin\frac{m\pi}{2l}(z+l)\,dz}{la^2\mu_i\,[J_1(\mu_i a)]^2}.$$

Simplifying and changing the limits of integration, and substituting

$$\varphi = \frac{m\pi}{2l}(z+l) \text{ which gives } dz = d\varphi\frac{2l}{\pi m}, \text{ and}$$

$$N_{im} = \frac{2\cdot 2l\int_{-\pi}^{\pi}(-1)\sin\varphi d\varphi}{\pi mla\mu_i J_1(\mu_i a)}.$$

Simplifying again and integrating

$$N_{im} = \frac{4\,[(-1)^m - 1]}{m\pi\mu_i a J_1(\mu_i a)} \text{ or}$$

$$N_{im} = \frac{4\,(\cos m\pi - 1)}{m\pi(\mu_i a)\,J_1(\mu_i a)} \tag{30}$$

[Thompson (*13*) sets $A_{\mu m} = -N_{im}$].

Therefore,

$$S = \sum_{i=1}^{\infty}\sum_{m=1}^{\infty} N_{im}J_o(\mu_i r)\sin\frac{m\pi}{2l}(z+l)\,e^{-k(\mu_i^2+\lambda_m^2)t}.$$

For a first term approximation: $m = 1$ and $\mu_i = \mu_1$

$$S = \frac{-8}{\pi\mu_1 a\,J_1(\mu_1 a)}J_o(\mu_1 r)\sin\frac{\pi}{2l}(z+l)\,e^{-k\left(\mu_1^2+\frac{\pi^2}{4l^2}\right)t}$$

and as $W = S + 1$ and from (20c),

$$v(r,z,t) = IT + (RT - IT)\,[W(r,z,t)] + \begin{cases} 0 & \text{for } t < t_1 \\ (TC - RT)\,W(r,z,t-t_1) & \text{for } t > t_1 \end{cases}$$

$$v(r,z,t) = IT + (RT - IT)\,[1 + S(r,z,t)] + \begin{cases} 0 \\ (TC - RT)\,[1 + S(r,z,t-t_1)] \end{cases}$$

$$v(r,z,t) = IT + (RT - IT) + (RT - IT)[S(r,z,t)] + \begin{cases} 0 \\ (TC - RT)[1 + S(r,z,t-t_1)] \end{cases} \tag{31}$$

$$= RT + (RT - IT)\left[-\frac{8}{\pi\mu_1 a J_1(\mu_1 a)}\, J_o(\mu_1 r) \sin\frac{\pi}{2l}\,(z+l)e^{-k\left(\mu_1^2 + \frac{\pi^2}{4l^2}\right)t} \right] +$$

$$\begin{cases} 0 \\ (TC - RT)\left[1 - \dfrac{8}{\pi\mu_1 a J_1(\mu_1 a)}\, J_o(\mu_1 r) \sin\dfrac{\pi}{2l}\,(z+l)e^{-k\left(\mu_1^2 + \frac{\pi^2}{4l^2}\right)(t-t_1)} \right] \end{cases}$$

where

$$\frac{8}{\pi\mu_1 a J_1(\mu_1 a)}$$

is Thompson's A_{11}.

Finally as $v(r,z,t)$ is the temperature at a point in the can $= CT$; for the first approximation:

$$CT = RT - (RT - IT)\left[A_{11} J_o(\mu_1 r) \sin\frac{\pi}{2l}\,(z+l)e^{-k\left(\mu_1^2 + \frac{\pi^2}{4l^2}\right)t} \right] +$$

$$\begin{cases} 0 \\ (TC - RT)\left[1 - A_{11} J_o(\mu_1 r) \sin\dfrac{\pi}{2l}\,(z+l)e^{-k\left(\mu_1^2 + \frac{\pi^2}{4l^2}\right)(t-t_1)} \right] \end{cases} \begin{cases} \text{for } 0 < t < t_1 \\ \text{for } (t > t_1)\ . \end{cases}$$

General solution

The general expression for the temperature at a point in the can is:

$$CT = RT - (RT - IT) \sum_{i=1}^{\infty} \sum_{m=1}^{\infty} A_{im} J_o(\mu_i r) \sin \lambda_m (z+l) e^{-k(\mu_i^2 + \lambda_m^2)t} + \tag{33}$$

$$\begin{cases} 0 \\ (TC - RT)\left[1 - \displaystyle\sum_{i=1}^{\infty} \sum_{m=1}^{\infty} A_{im} J_o(\mu_i r) \sin \lambda_m (z+l) e^{-k(\mu_i^2 + \lambda_m^2)(t-t_1)} \right] \end{cases} \begin{cases} \text{for } 0 < t < t_1 \\ \text{for } t > t_1 \end{cases}$$

where CT, can temperature at a point; RT, retort temperature (or processing steam temperature); IT, initial temperature throughout the can; and TC, cooling bath temperature; and temperatures can be measured either in °F. or °C. as long as consistent. $A_{im} = -N_{im}$ is a constant as determined in (30) and depending on the initial temperature distribution in the can, assumed to be uniform, and on the dimensions of the can.

$$-N_{im} = \frac{-4\,[(-1)^m - 1]}{m\pi(\mu_i a) J_1(\mu_i a)} = \frac{4\,(1 - \cos m\pi)}{m\pi(\mu_i a) J_1(\mu_i a)}$$

$J_o(\mu_i r)$ is the Bessel function of first kind, zero order which can be found in tables of Bessel cylindrical functions, and which is equal to 1 in the center at $r = 0$.

$J_1(\mu_i a)$ is the Bessel function of first kind, first order and

$(\mu_i a)$ = positive zeros infinite in number of $J_o(\mu_i a) = 0$ or

μ_i = positive roots infinite in number of $J_o(\mu_i a) = 0$

all of which can be found in tables of Bessel functions and t is time; t_1 is time when heating is terminated and cooling starts; r,z are cylindrical coordinates of space with the origin in the center of the can; a is radius of the can; $2l$ is total height of the can; k is thermal diffusivity; and λ_m is $\frac{m\pi}{2l}$.

Note that sin λ_m (z + l) = 1 in the center of the can, which results in $A_{im} = -N_{im}$ equal to Ball's (*1*),(*2*) *j* at the center of the container where $J_o(\mu_i r) = 1$.

SUMMARY

A theoretical relationship has been derived between time, temperature, and space in a cylindrical container. Starting with the flow of heat through a very small but finite elementary volume, Fourier's general equation of heat conduction was derived (12), then transformed into cylindrical coordinates (14a) assuming two-dimensional flow: radial and axial. The thermal diffusivity in an isotropic homogeneous body was assumed constant and the same in all directions. Equation 14a was then solved assuming an initial temperature distribution uniform throughout the container, and assuming also instantaneous rise of the temperature at the boundaries to the processing temperature (of the steam or cooling water). In order to solve Equation 14a it was necessary to transform it to obtain substantially a temperature scale from 0 to 1, thus obtaining homogeneous boundary conditions. The equation was solved by the method of separation of the variables and the general solution obtained was a linear combination of products of particular solutions in the form of Bessel functions, trigonometric functions and exponentional functions. The constants of the relationship were determined by means of a Fourier-Bessel expansion Equation 30. Expressions were then obtained for the first approximation and for the general solution in terms of a double series Equations 32 and 33 respectively, which permit determination of the temperature at a point within a cylindrical container at any time during the thermal process.

The general expression for the temperature at a point in the can is given in Equation 33.

LITERATURE CITED

1. Ball, C. O. Thermal process time for canned foods. Nat. Research Council (U.S.), *Bull.*, 37 (1923).
2. Ball, C. O. Mathematical solution of problems on thermal processing of canned foods. Univ. Calif. (Berkeley), *Pubs. Public Health*, 1 (2), 15 (1928).
3. Carslaw, H. S. Introduction to the Theory of Fourier's Series and Integrals. 1921, MacMillan and Company, London.
4. Carslaw, H. S., and Jaeger, J. C. Operational Methods in Applied Mathematics. 1941, Oxford University Press.
5. Churchill, R. V. Fourier Series and Boundary Value Problems. 1941, McGraw-Hill Book Co., Inc., New York.
6. Fourier, Joseph. The Analytical Theory of Heat (Trans. from French). 1878, Cambridge, at the University Press.
7. Hicks, E. W. On the evaluation of canning processes. *Food Technol.*, 5, 134 (1951).
8. Ingersoll, L. R., Zobel, O. J., and Ingersoll, A. C. Heat Conduction. 1948, McGraw-Hill Book Co., Inc., New York.
9. Jackson, J. M., and Olson, F. C. W. Thermal processing of canned foods in tin containers. IV. Studies of the mechanisms of heat transfer within the container. *Food Research*, 5, 409 (1940).
10. Merrill, D. G. Heating rates of food in glass and other containers. *Ind. Eng. Chem.*, 40, 2263 (1948).
11. Olson, F. C. W., and Schultz, O. T. Temperatures in solids during heating and cooling. Tables for the numerical solution of the heating equation. *Ind. Eng. Chem.*, 34, 874 (1942).
12. Reddick, H. W. Differential Equations. 1947, John Wiley and Sons, Inc., New York.
13. Thompson, G. E. Temperature-time relations in canned foods during sterilization. *J. Ind. Eng. Chem.*, 7, 657 (1919).

CHARACTERIZATION OF THE EFFECT OF FREEZING ON COOKED EGG WHITE [a,b]

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It has been known for some time that hard cooked eggs cannot be used satisfactorily in food mixtures, such as chicken a la king, that are to be frozen, because the white becomes rubbery, granular, watery, and separates into small clumbs or layers *(1)*. Yet the effect of freezing on cooked egg white has not been characterized in terms of factors, such as time and temperature of cooking, freezing method, pH of the white, or the effect of additives that might influence the character of the product. It has been the principal object of our present investigation to characterize the effect of freezing cooked egg white in terms of these rather obvious factors. A solution to the problem has not become evident, but the results appear to be of some fundamental interest and should provide a basis for any future work on this subject that might be undertaken.

Since for practical purposes cooked yolk is unchanged by freezing *(1)*, this report principally concerns the appearance and texture of hard cooked egg white subjected to freezing. The results show that the quality of the cooked egg white varies most markedly with factors that affect ice crystal size. Thus, large ice crystals leave large holes or clefts. This is especially pertinent since no appreciable amount of the water frozen out of the coagulum structure is taken up by the coagulum on thawing. The practically irreversible water separation on freezing is perhaps the principal bar to finding a treatment or combination of treatments that consistently gives completely satisfactory frozen egg white.

Although not directly connected with the problem of frozen cooked white, data from a limited number of experiments indicate that mixtures of egg yolk and white might satisfactorily be used for certain food products.

METHODS AND PROCEDURES

White from fresh grade AA eggs was mixed at moderate speed in a Waring blendor controlled through a variable transformer. Air inclusion was kept at a minimum by holding a small flask about half submerged in the liquid to prevent the formation of a vortex. Mixing was discontinued as soon as the white appeared homogeneous. Additives, when used, were included at this point. The pH was adjusted with the use of a glass electrode with 2 *N*. acetic acid or anhydrous sodium carbonate powder. The acid or alkali was added slowly with stirring to minimize local concentrations without diluting the egg white excessively by use of dilute solutions. After mixing, the white was centrifuged in approximately 60 ml. portions to remove air. If the mixing was incomplete, it was apparent after centrifugation by the presence of a gelatinous layer of thick white

[a] Presented at the Twelfth Annual Meeting of the IFT, Grand Rapids, Mich., June 11, 1952.

[b] Report of a study made under the Research and Marketing Act of 1946.

[c] Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture.

in the bottom of the tube. To obtain samples of uniform size and rate of heat transfer during cooking and freezing, 50 ml. of the centrifuged white was pipetted into trays 3 inches square by 1 inch deep made of 0.0015-inch aluminum foil. This amount of white gave a layer about ¼ inch deep.

Internal temperature of the samples was determined with copper-constantan thermocouples of 24 B and S gage wire with soldered junctions 1 mm. long placed in the center of the sample about midway between the top and bottom of the layer and held in place with a wire frame and spring clip. The location of the thermocouple was not critical so long as the junction was submerged and not touching the bottom of the tray.

The samples, with the thermocouples in place, were cooked in steam at atmospheric pressure. The cooker consisted of two parts made from stainless steel. The base was of 8-mesh wire cloth supported by a 1-inch-high frame around the edge. The cover, which fitted down over the base, was pyramidal with an opening about 1 inch square at the top to allow steam to escape. The pyramidal shape was used to allow condensed water to drain down the sides rather than to drip back into the samples. The cooker was preheated on a boiling steam bath. When hot, the cover was lifted, the samples placed on the base and the cover replaced. The steam was shut off during the placing of the samples and turned on again as soon as they were in place. The time of cooking was taken from the time the steam was turned on until it was shut off and the cooked samples removed. Except for the study of effect of cooking time, all samples were cooked for 3 minutes.

In those experiments concerned with the possible utility of mixtures of yolk and white, the eggs were separated, the yolks and whites separately blended, recombined in the desired proportions and cooked by the above method. The cooked mixture was diced, mixed into a white sauce and frozen at —10°F.(—23°C.). After thawing and heating, the samples were examined subjectively for taste and texture.

After cooking, the samples to be frozen were suspended in ½-gallon friction-top cans which had been painted inside and out with flat black to assist heat transfer. The purpose of the cans was to reduce temperature fluctuations from drafts caused by opening and closing the doors of the freezing rooms. The thermocouple leads were passed through a hole in the lid of the can and connected to a multiple-point temperature-recording potentiometer on which the freezing curves were recorded.

After freezing overnight, the samples were divided into 2 parts while still frozen. One part was set aside to thaw and the other part again divided, cut into pieces and put into tared 50-mesh monel wire cups. These cups were constructed with a solid rim at the top so that they fitted into and were suspended in the upper halves of 80 ml. plastic centrifuge tubes. The loaded wire cups were weighed and placed in tared tubes and centrifuged at half rheostat setting in a Servall angle-head centrifuge (ca. 2400 r.p.m.). The liquid obtained was weighed and calculated as percent of free liquid. The difference between duplicate determinations averaged about 6% of the amount of free liquid found.

The thawed half of the sample was carefully cut into uniform strips ¼ inch wide. Four strips were laid together with the cut surfaces uppermost and photographed at 5× magnification under oblique light. This method of recording the structural appearance of samples was chosen for the sake of simplicity as compared to sectioning and photographing frozen samples. Since the differences of structure were readily apparent when the frozen samples were cut, the possible changes occurring during thawing had no practical significance. The structure in all samples, whether coarse or fine, was uniform throughout the sample.

RESULTS

Figure 1 shows the marked degree of porosity obtained when cooked egg white is frozen. Section A is an unfrozen control which shows no porosity at this magnification and Sections B, C, and D show the range of porosity obtained as a result of freezing.

In the coarse-structured samples, as illustrated by Section D, the voids form fairly continuous openings to the surface. Where the structure is less coarse, as in C, there are fewer channels to the surface while in the finest samples, as in B, the liquid seems to be distributed in approximately globu-

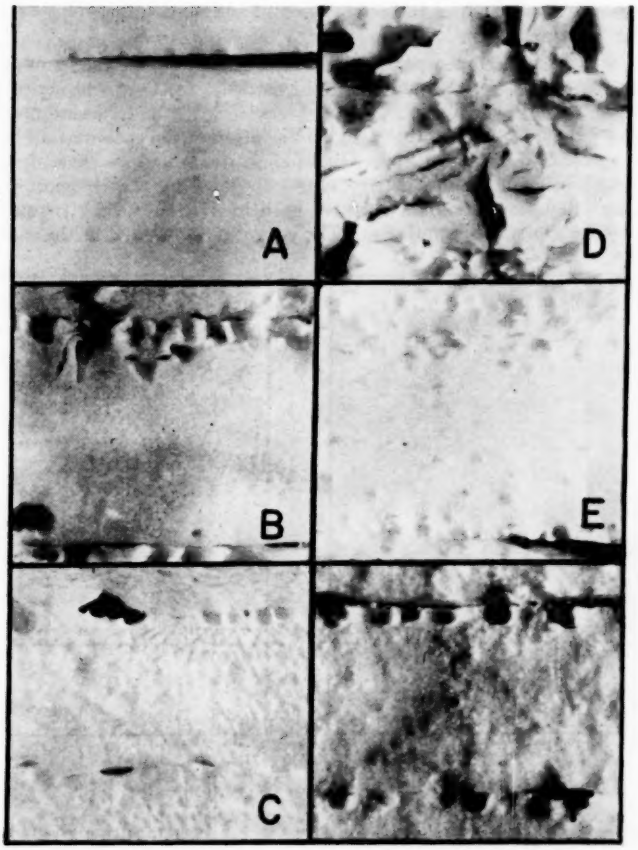

Figure 1. Frozen cooked egg white (5 ×). A, Unfrozen control. B, pH 7.6, supercooled 16°F., 41% liquid separation. C, pH 7.4, supercooled 6°F., 58% liquid separation. D, pH 7.8, supercooled 1°F., 60% liquid separation. E, Unfrozen, showing gas bubbles. F, Crumbly, fine-grained structure characteristic of addition of solid phase (calcium carbonate).

lar cells. It is reasonable to suppose, therefore, that the actual differences in amount of free liquid are less than those found by centrifugation and that the amount of liquid found in this way is to some extent a measure of average size and extent of interconnection of the liquid-filled spaces formed during freezing.

Effect of time and temperature of cooking. In the apparatus used, 2½ minutes in atmospheric steam was the least time for complete coagulation of the white and over 5 minutes caused discoloration and formation of gas bubbles particularly around the edges. Variation of the cooking time within the range of 2½ to 5 minutes in atmospheric steam had no effect upon porosity or upon the amount of free liquid obtained by centrifugation after freezing. Samples cooked in a water bath at 176°F.(80°C.) required 25 minutes for complete coagulation. The coagulums obtained at the different

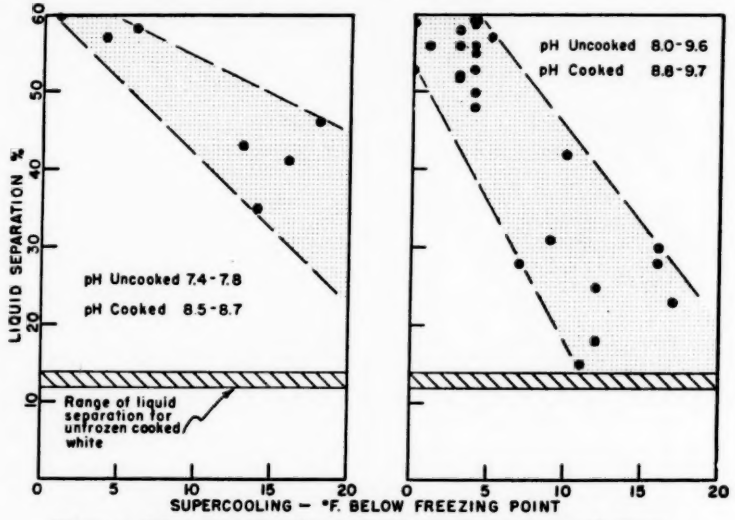

Figure 2. Effect of supercooling on liquid separation in two pH ranges.

temperatures were cooked sufficiently to be quite firm although they were not quite identical in appearance. After freezing the damage was essentially the same to the coagulums obtained at either temperature. Thus, limited variation in the temperature of coagulation apparently does not affect the separation of water from the coagulum during freezing or its return after thawing.

Effect of manner of freezing. The rate of freezing and the degree of supercooling, as altered by temperature or other means, was found to influence the appearance of the frozen and thawed egg white. The liquid separation on centrifugation is given in Figure 2 as a function of the degree of supercooling. Since the degree of supercooling will vary with the rate of cooling, such curves as those shown in Figure 3 can be obtained. The

smaller amount of free liquid obtained in the range 0° to 10°F.(−18° to −12°C.) correlates with observations made in other experiments with our equipment that supercooling is greatest in this temperature range.

The rapidity of crystallization in the supercooled egg white was shown in one temperature recording of a sample that had supercooled 15°F.(8°C.) below the freezing point. Crystallization was initiated while the print wheel of the recorder was in contact with the chart and the temperature returned to the normal freezing temperature of 30°F.(−1°C.) while the wheel was still in contact. With the recorder used, the total time the print wheel is in

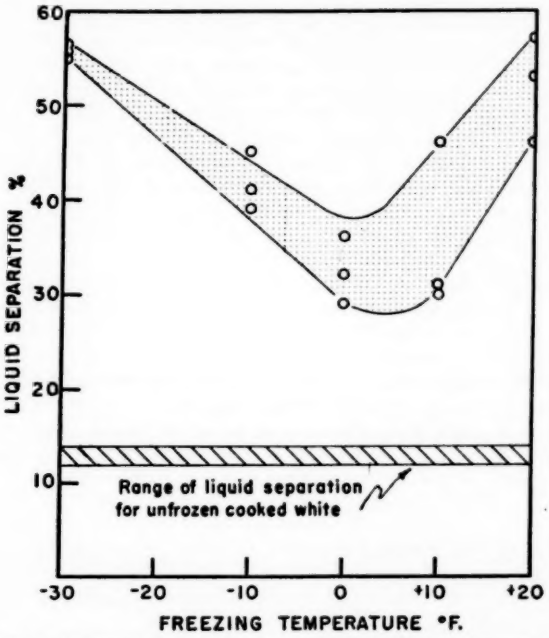

Figure 3. Apparent effect of freezing temperature on liquid separation.

contact with the strip chart is between 1 and 2 seconds so that, at least in the region of the thermocouple, crystal formation took place within a fraction of this time.

Effect of pH. The pH of the uncooked white was adjusted to various levels over the range of pH 6.0 to 10.2. Figure 4 shows the pH after cooking as a function of the pH before cooking for both fresh liquid and lyophilized egg white. The difference is probably due to loss of carbon dioxide during lyophilization. The appearance of the cooked white is shown in Figure 5. For the study of freezing effects, the range was restricted to pH 7.0 to 9.5 because of the curdled, chalky appearance at lower pH and the rubbery translucence at higher pH.

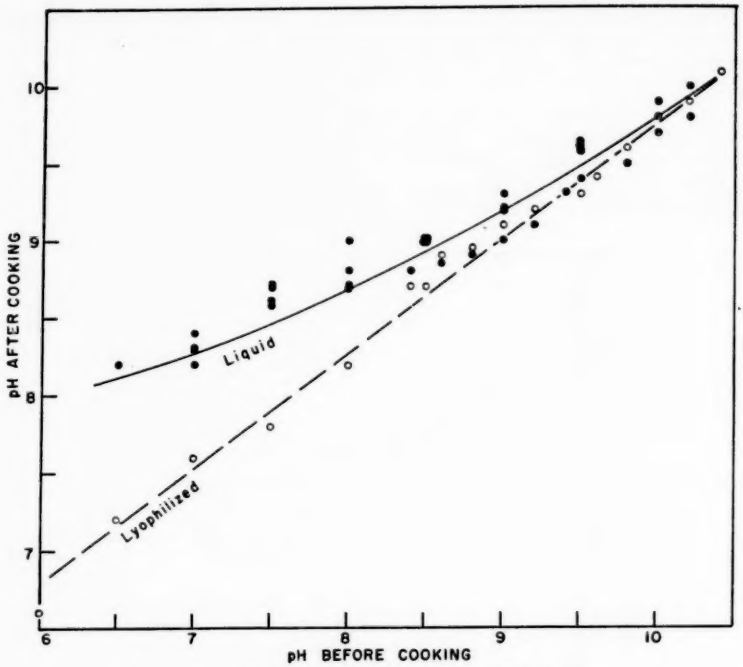

Figure 4. Relation between pH before and after cooking.

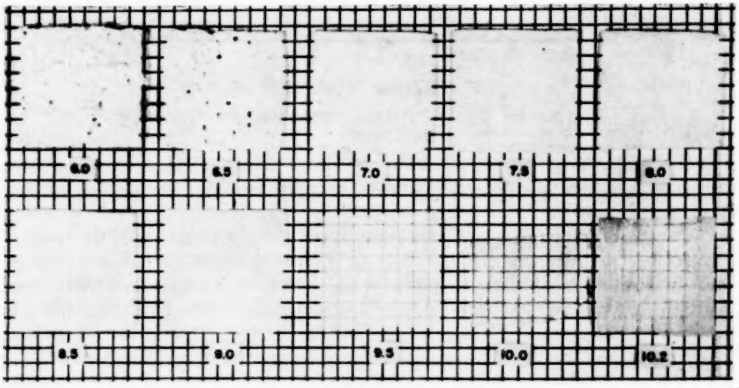

Figure 5. Effect of pH on appearance of cooked egg white.

There was generally greater liquid separation after freezing at the lower pH values (Figure 2). However, the appearance is to some extent independent of the liquid separation so that for a given degree of supercooling, improved appearance and high liquid separation can occur in the same sample (Figure 1, B and C). The effect of pH did not seem sufficient to warrant further studies.

Effect of finely dispersed solids. Barium sulphate, magnesium oxide, and calcium carbonate were added to egg white in an attempt to get small ice crystals by providing many nuclei for the formation of ice crystals. Magnesium oxide and barium sulphate were both unsatisfactory because they caused a massive precipitate to form rather than allowing the small particles to be dispersed in the liquid egg white. Calcium carbonate, however, disperses readily and remains reasonably well suspended. Calcium carbonate can also be precipitated in the egg white by the addition of a soluble calcium salt, which reacts with the carbonate already present in the white. The latter method of introducing solid nuclei is preferable because it helps to reduce gas bubble formation during cooking.

The carbon dioxide present in the egg white and that formed by the decomposition of bicarbonate during heating tend to come out of solution during cooking and to form gas pockets as shown in Figure 1 E. This tendency is much more pronounced in the presence of a solid phase, such as calcium carbonate, which acts as nuclei for gas bubble formation as well as for ice crystal formation. When sufficient calcium is added to combine with all the carbonate present in the white, the formation of gas pockets during cooking is prevented and the porosity after freezing is barely apparent (Figure 1 F). However, the gel is weak and crumbly and tastes like chalk. A stepwise addition of calcium with removal of the initial precipitate by centrifugation before cooking results in a better structure, but does not satisfactorily reduce the chalk taste.

Effect of soluble additives. When added to egg white, water, sodium chloride, glucose, glycerine, and the vegetable gums tragacanth, karaya, locust, ghatti, and acacia gave no improvement of structure after cooking, freezing, and thawing. The addition of gelatin, in some cases, gave considerable improvement of structure. However, gelatin addition increased the frequency of supercooling and this appears to be the principal reason for the improvement, which is not obtained consistently because of the nature of the phenomenon of supercooling.

Mixtures of yolk and white. These mixtures were examined subjectively only for their possible utility in frozen food mixtures.

During and immediately following cooking, the yellow color of the mixtures of yolk and white underwent a change which was strongly affected by pH. Below pH 7.5 to 8.0 this change, although apparent, was not objectionable. Above pH 8.0 the greenish coloration became strong enough to render the samples unacceptable. The mixtures also became increasingly rubbery as the pH was increased. Milk and water were added in an attempt to improve the tenderness. The milk gave little or no improvement, but the addition of 20% water resulted in marked improvement of tenderness.

In general, it was agreed by a number of persons who examined the samples that a yolk:white ratio from 40:60 to 80:20, when diluted with

20% water and adjusted to pH 6.0 to 7.0 before cooking, would be suitable for inclusion in frozen food mixtures.

DISCUSSION

The results make it appear that freezing cooked egg white causes a series of events such as the following: During freezing the water in the elastic gel of cooked egg white (denatured protein) migrates to increase the size of ice crystals wherever nuclei are present. As the crystals grow, they penetrate the gel and separate the structure, thus releasing a part of the elastic tension. The migration of the water from within the gel structure plus the force exerted by the growth of ice crystals and the release of elastic tension by mechanical cleavage cause the gel structure to contract. That this contraction is largely irreversible is demonstrated by the liquid-filled spaces remaining after thawing. The structure remaining is naturally tougher, since it contains a considerably higher proportion of protein than the original gel. Thus, if the gel before freezing contains 12% solids and if a 55% liquid separation occurs during freezing, as occurred in many experiments, then the remaining gel would contain upward to 27% solids. The actual remaining solids will be somewhat lower than 27% because the liquid will contain a part of the soluble solids.

An obvious way of minimizing the disruption of the cooked egg white structure would be to produce and maintain small ice crystals on freezing. Supercooling leads to small crystals. Gelatin seems to increase the frequency of supercooling somewhat. Freezing at 0° to 10°F.(−18° to −12°C.) is the optimum temperature zone for supercooling of cooked egg white.

The effect of pH on the amount of liquid separable by centrifuging thawed white may be related to the tendency of the gel to be finer in structure (less opaque) and to be stronger at the higher pH levels. Thus, relative opacity is a qualitative measure of the submicroscopic coarseness of a gel structure and of its tendency toward syneresis *(2)*. Actually, the same amount of ice may form at the different pH values, but the strength of the gel may merely prevent its escape from some of the small size "pockets" on centrifugation.

A strengthening of the gel is not the explanation for improvement in appearance caused by addition of calcium carbonate. Such gels actually tend to be weaker than ordinary gels so that the improvement would appear to be due to the numerous particles of calcium carbonate acting as sites for the formation of numerous small ice crystals.

The products obtained from mixed yolk and white had more of the characteristics of yolk than of white and would therefore more suitably represent yolk than white in a food product. Such mixtures have the advantage of being more cohesive than egg yolk separated from hard cooked eggs and are therefore better able to withstand mixing or stirring.

SUMMARY AND CONCLUSIONS

The damage to cooked egg white caused by freezing appears to be due to the mechanical effects of the ice crystals formed. The damage is reduced by factors which tend to promote small crystal formation, such as supercooling or the adition of finely divided calcium carbonate.

Soluble additives, with the possible exception of gelatin, did not significantly influence the response of the egg white to freezing. Lowering the pH did not affect the liquid separation significantly unless the egg white was supercooled prior to freezing. When they were supercooled, the samples at the lower pH yielded more liquid on centrifugation. However, none of these factors that influenced the quality of frozen and thawed cooked egg white either separately or in combination are considered to yield cooked egg white that behaves consistently satisfactorily on freezing.

The results of limited studies of mixtures of egg yolk and white indicate that an egg preparation suitable for use in frozen foods could be prepared from such mixtures.

LITERATURE CITED

1. Woodroof, J. G. Problems in freezing cooked foods. *Quick Frozen Foods*, **8** (9), 90 (1946).

2. Jirgensons, B. The gelatinization of albumin in salt-containing aqueous propyl alcohol-thixotropy and syneresis of the albumin-propyl alcohol gel. *Kolloid Z.*, **74**, 300 (1936) *C. A.* **30**, 5096 (1936).

EFFECT OF GERMINATION ON THE TOTAL TOCOPHEROL CONTENT OF PULSES AND CEREALS

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(Manuscript received June 6, 1952)

In our studies, we observed that during the process of germination there occurred an increase in the levels of nicotinic acid *(7)*, ascorbic acid *(6)*, thiamine *(1)*, riboflavin *(8)*, carotene *(2)*, and choline *(3)* in different varieties of pulses and cereals. Tocopherol (vitamin E) is known to be present in plant foods *(5)*. Tocopherol values of pulses and cereals were, therefore, determined both before and during the course of germination.

EXPERIMENTAL

Seeds were germinated as described previously *(3)*. After varying periods of germination they were removed, dried, and crushed in a glass mortar. Then 5-g. aliquots were taken in an Erlenmeyer flask to which 3 ml. of 5% pyrogallol was added, and the contents were saponified by refluxing with 2 *N*. methanolic potassium hydroxide on a boiling water bath for 30 minutes in an atmosphere of nitrogen. The saponified material was extracted several times with petroleum ether and the combined ether extracts were washed first with a 2% solution of potassium hydroxide and subsequently with several changes of distilled water to make the extract free from alkali. The ether solution was dried over anhydrous sodium sulfate. To make the extract free from carotenoids, it was treated with 85% sulfuric acid and the centrifuged ether extract, after washing with alkali and water, was dried with anhydrous sodium sulfate. The ether was evaporated in an atmosphere of nitrogen and the residue was dissolved in 50 ml. of a mixture of benzene and ethanol (1 to 1). The total tocopherol content of the pulses was estimated chemically according to the method of Emerie and Engel *(4)* slightly modified. The results are given in Table 1.

TABLE 1

Effect of germination on the tocopherol content of pulses and cereals

Botanical name	Tocopherol content[a]		
	Ungerminated	After germination for	
		2 days	4 days
	mg. per 100 g.	*mg. per 100 g.*	*mg. per 100 g.*
Phaseolus radiatus	2.4	2.8	3.2
Cicer arietinum	2.0	2.3	2.6
Phaseolus mungo	1.9	2.4	2.5
Lens esculenta	2.0	2.3	2.5
Pisum sativum	2.2	2.4	2.7
Cajunus indicus	1.6	1.8	2.1
Phaseolus roxburgi	1.5	1.7	1.9
Triticum vulgare	2.2	2.3	2.5
Oriza sativa	2.3	2.4	2.6
Zea mays	1.8	1.9	2.0

[a]Average of 6 determinations.

In order to verify the accuracy of the method used, known amounts of tocopherol were added to 10 g. of crushed *Phascolus radiatus* and the tocopherols were then extracted and estimated as described above. Good recovery of added tocopherol was obtained (Table 2).

TABLE 2

Recovery of tocopherol added to 10 g. of pulse (Phaseolus radiatus)

Sample No.	Tocopherol added	Tocopherol estimated	Tocopherol recovered
	mg.	*mg.*	%
1	0	0.24	....
2	0.5	0.74	94
3	1.0	1.20	97
4	1.5	1.64	94
5	2.0	2.18	97
6	3.0	3.15	97

RESULTS AND DISCUSSION

Pulses and cereals contain a good amount of tocopherols and these values are increased in some plants during the progress of germination. The method of estimation of tocopherols employed in the present investigation does not, however, furnish data on the amount of the biologically active alpha-tocopherol which is present. Increased tocopherol values of germinated pulses, along with the increased values of other vitamins *(1, 2, 3, 6, 7, 8)*, make germinated pulses nutritionally superior to ungerminated pulses and, in countries like India, where pulses form an important component of the diet, the consumption of germinated pulses should be encouraged.

SUMMARY

Both pulses and cereals contain a good amount of tocopherols. The tocopherol contents of pulses are increased during germination. Germination in cereals causes a smaller increase in their tocopherol values.

LITERATURE CITED

1. Chattopadhyay, H., Nandi, N., and Banerjee, S. Effect of germination on the thiamine content of pulses grown in Bengal. *Indian Pharmacist*, **5**, 126 (1950).
2. Chattopadhyay, H., and Banerjee, S. Effect of germination on the carotene content of pulses and cereals. *Science*, **113**, 600 (1951).
3. Chattopadhyay, H., and Banerjee, S. Studies on the choline content of some common Indian pulses and cereals both before and during the course of germination. *Food Research*, **16**, 230 (1951).
4. Emmerie, A., and Engel, C. Colorimetric estimation of alpha-tocopherol (vitamin E). *Rec. trav. chim.*, **57**, 1351 (1938).
5. Harris, P. L., Quaife, M. L., and Swanson, W. J. Vitamin E content of foods. *J. Nutrition*, **40**, 367 (1950).
6. Nandi, N., and Banerjee, S. Effect of germination on the vitamin C content of pulses grown in Bengal. *Indian Pharmacist*, **5**, 63 (1949).
7. Nandi, N., and Banerjee, S. Effect of germination on the nicotinic acid content of pulses grown in Bengal. *Indian Pharmacist*, **5**, 13 (1949).
8. Nandi, N., and Banerjee, S. Effect of germination on the riboflavin content of pulses grown in Bengal. *Indian Pharmacist*, **5**, 202 (1950).

THE TASTE INTERRELATIONSHIP OF MONOSODIUM GLUTAMATE AND SUCROSE [a,b]

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(Manuscript received June 19, 1952)

In a previous paper Lockhart and Gainer *(4)* summarized the results in the earlier literature pertaining to the monosodium glutamate (MSG) threshold and the effects of MSG on primary taste sensations. They also reported that subliminal concentrations of MSG had no significant effect on the sweetness of low concentrations of sucrose.

The purpose of the present study, the findings of which are presented below, was twofold: first, to determine the threshold values of MSG related to a series of more concentrated sucrose solutions, and second, to determine whether sweetness of more concentrated sucrose solutions is enhanced or depressed either by subliminal or by supraliminal concentrations of MSG.

EXPERIMENTAL PROCEDURE

The experimental design employed in this study was somewhat different from that described in the previous paper. For convenience, a brief summary of procedure is given here.

The taste panel was composed of judges selected from the departmental staff. Both men and women participated. Some were smokers, others were not. All had previous experience in judging food quality, in threshold testing, and in other studies involving MSG.

The tests were conducted in mid-morning and mid-afternoon, at least one hour after meals.

The test solutions were presented as an asymmetric binominal system of the type AA-B, as described by Lockhart *(3)*. This system is more familiarly known as the "triangle test," the phrase used by Helm and Trolle *(2)*. The solutions, in 25-ml. portions, were served in identical coded beakers. Each set of 3 consisted either of 1 sample of pure sucrose and 2 of sucrose plus MSG, or of 2 samples of sucrose and 1 of the mixture. Combinations were presented randomly, and judges were never given any information about the nature of the combination, except that 2 of the 3 samples were alike. As usual, each judge was asked to select the odd sample. In addition, each was asked to select the sweeter of the two groups and also to state whether the taste in either group was different from that of pure sucrose, without defining the difference, if any existed.

Several series of experiments were carried out. Each individual test consisted of a comparison of a sucrose solution of specific concentration, taken as a standard, and a mixed solution containing the standard amount of sucrose and some MSG. The amount of MSG in the mixture was varied from test to test. A summary of the experimental program follows:

Series 1. Distilled water versus:
- a. distilled water plus 0.0010 *M.* MSG
- b. distilled water plus 0.0015 *M.* MSG
- c. distilled water plus 0.0025 *M.* MSG

---

[a] Presented at the Twelfth Annual Meeting of the IFT, Grand Rapids, Mich., June 11, 1952.

[b] Contribution No. 135 from the Department of Food Technology, Massachusetts Institute of Technology.

Series 2. Sucrose, 0.036 *M.*; versus:
- a. sucrose, 0.036 *M.*; plus 0.0010 *M.* MSG
- b. sucrose, 0.036 *M.*; plus 0.0015 *M.* MSG
- c. sucrose, 0.036 *M.*; plus 0.0025 *M.* MSG

Series 3. Sucrose, 0.072 *M.*; versus:
- a. sucrose, 0.072 *M.*; plus 0.0010 *M.* MSG
- b. sucrose, 0.072 *M.*; plus 0.0015 *M.* MSG
- c. sucrose, 0.072 *M.*; plus 0.0025 *M.* MSG
- d. sucrose, 0.072 *M.*; plus 0.0035 *M.* MSG

Series 4. Sucrose, 0.144 *M.*; versus:
- a. sucrose, 0.144 *M.*; plus 0.0020 *M.* MSG
- b. sucrose, 0.144 *M.*; plus 0.0025 *M.* MSG
- c. sucrose, 0.144 *M.*; plus 0.0040 *M.* MSG
- d. sucrose, 0.144 *M.*; plus 0.0050 *M.* MSG

Series 5. Sucrose, 0.288 *M.*; versus:
- a. sucrose, 0.288 *M.*; plus 0.0025 *M.* MSG
- b. sucrose, 0.288 *M.*; plus 0.0035 *M.* MSG
- c. sucrose, 0.288 *M.*; plus 0.0045 *M.* MSG
- d. sucrose, 0.288 *M.*; plus 0.0055 *M.* MSG
- e. sucrose, 0.288 *M.*; plus 0.0075 *M.* MSG

The concentrations of MSG employed in the mixtures listed above were selected after preliminary experimentation had indicated that the odd sample in any test would be identified correctly more than 33% of the time, but less than 100% of the time.

The data were derived from the efforts of 8 judges, who repeated each test until at least 21 judgments were obtained. That portion of the data relating to the identification of the odd sample is recorded in Table 1. That relating to the relative sweetness of the

TABLE 1

Judgments in triangle tests with pure solutions of sucrose and mixed solutions containing monosodium glutamate

Sucrose in pure solution	MSG in mixed solution	Judgments			Percentage	
		Total	Correctly identified	Adjusted [c] score	Adjusted score	Above [d] chance
M.	*M.*					
0.000	0.0010*	24	12	12.0	50.0	25.0
	0.0015*	24	16	16.0	66.7	50.0
	0.0025*	24	22	22.0	91.8	87.6
0.036 (1.25%)	0.0010	24	10	10.0	41.7	12.6
	0.0015	24	13	13.3	55.4	33.2
	0.0025	24	20	20.0	83.3	75.0
0.072 (2.5%)	0.0010	24	8	9.0	37.5	6.3
	0.0015	47	19	20.7	44.1	16.2
	0.0025	67	43	44.0	65.7	48.6
	0.0035	24	20	20.0	83.3	75.0
0.144 (5.0%)	0.0020	21	8	8.7	41.4	12.2
	0.0025	47	26	28.0	59.6	39.5
	0.0040	21	14	14.0	66.7	50.2
	0.0050	24	20	20.0	83.3	75.0
0.288 (10.0%)	0.0025	47	21	22.0	46.8	20.2
	0.0035	22	10	10.7	48.7	23.1
	0.0045	46	24	24.3	52.9	29.4
	0.0055	21	12	13.3	63.3	45.0
	0.0075	22	19	19.0	86.3	79.5

[c] Correctly identified judgments plus ½ of the number of no-difference judgments.
[d] Calculated from formula: % above chance = 3/2 (% observed − % expected).
* Data from Lockhart and Gainer (*4*).

TABLE 2

Sweetness and taste judgments of correct identifications

Sucrose in pure solution	MSG in mixed solution	Correct identifications	Mixed solutions			
			Less sweet	Chi[f] square	Taste different	Chi[f] square
M.	*M.*					
0.036	0.0010	9	7.0	2.78	7.5	4.00[g]
(1.25%)	0.0015	13	9.5	2.78	11.5	7.70[g]
	0.0025	20	13.0	1.80	19.0	16.20[g]
0.072	0.0010	8	4.0	0.00	4.0	0.00
(2.5%)	0.0015	19	10.5	0.21	14.0	4.26[g]
	0.0025	43	31.0	8.37[g]	37.0	22.30[g]
	0.0035	20	16.5	8.50[g]	16.0	7.20[g]
0.144	0.0020	8	4.5	1.12	7.0	4.50[g]
(5.0%)	0.0025	26	16.0	1.38	22.0	12.47[g]
	0.0040	14	12.0	7.14[g]	13.0	10.28[g]
	0.0050	20	17.0	9.80[g]	18.5	14.40[g]
0.288	0.0025	21	10.0	0.05	12.5	0.76
(10.0%)	0.0035	10	4.5	0.10	7.0	1.60
	0.0045	24	14.5	1.04	20.0	10.67[g]
	0.0055	12	4.5	0.90	8.5	2.08
	0.0075	19	15.0	0.84	16.5	10.03[g]

[f] Chi square = 3.841 at P = 0.05 level: Snedecor *(5)*.
[g] Significant at P = 0.05.

two kinds of samples in any one test and that indicating a change in the character of the taste are recorded in Table 2.

RESULTS AND DISCUSSION

Table 1 shows that the number of odd samples correctly identified was recorded as the test score. In some cases in which several judges were unable to select any sample as being different from the other two, one-third of the "no difference" judgments was added to the number of correct identifications of the odd sample to give an adjusted score. The numerical scores were then transformed into percentage scores.

When no detectable sensory difference exists, it is probable that a chance percentage of correct identifications equal to 33.3 will be scored by a panel working with a triangle system. Consequently, it is more meaningful to calculate the percentage-above-chance scores. This was done by applying the formula:

$$P_C = 3/2\ (P_O - P_E)$$

where P_C is the percentage above chance; P_O the percentage observed; and P_E is the percentage expected.

For P_E equal to 33.3, the formula becomes $P_C = 3/2\ (P_O - 33.3)$.

The percentage score and the percentage-above-chance score obtained for each test are also shown in Table 1.

Harrison and Elder *(1)* have indicated how information derived by the technique described above may be presented graphically. These authors recommend that concentrations (in this case those of MSG used in the mixtures) be plotted against percentage-above-chance scores on log-probability

paper. They explain that "this choice of coordinates is taken because flavor perception is expected to vary with the logarithm of the stimulus value (Weber-Fechner Law) and the distribution of scores around the halfway point is assumed to follow the normal probability function." As shown in Figure 1, the data plotted on log-probability paper give a family of straight lines, one for each standard sucrose concentration and one for the series in which distilled water was used as the standard.

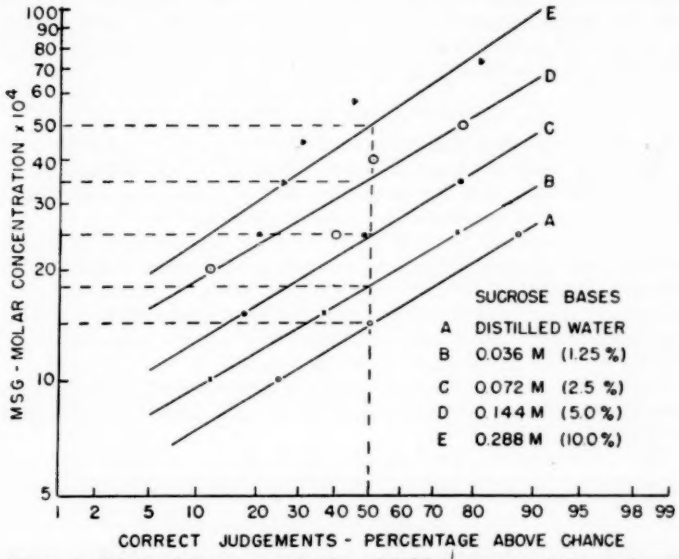

Figure 1. Relation between concentration of MSG in sucrose solution and correct identification of odd sample in triangle test, scored as the percentage of judgments above chance.

The threshold concentration of MSG in any one of the standard solutions is the concentration that corresponds to a percentage-above-chance of 50. For distilled water the MSG threshold was found to be 0.00143 *M.*; for 0.036 *M.* sucrose, 0.0018 *M.*; for 0.072 *M.* sucrose, 0.0025 *M.*; for 0.144 *M.* sucrose, 0.0035 *M.*; and for 0.288 *M.* sucrose, 0.0050 *M.* It is obvious that as the concentration of sucrose is increased, the concentration of MSG in a mixture required to produce a detectable difference in taste must also be increased. In this case, when the concentration of sucrose is doubled, the factor of increase for MSG is approximately 1.4. With this factor it is possible to predict the threshold concentration of MSG that is associated with any concentration of sucrose.

In Table 2 are recorded the number of correct identifications of the odd sample, the number of judgments that the samples containing MSG were less sweet, and the number of judgments that the taste of the mixed solution was different in character from true sweetness.

A casual examination of the column headed "less sweet" would give, perhaps, the impression that the mixed solutions were not so sweet as the solutions containing only sucrose. However, after the chi square test was applied to each result, only 4 of the 16 values were found to be significant. Here again is another illustration of the fact that generalizations should not be made without support from statistical analysis.

If the results derived from the taste-different-from-sweetness judgments are analyzed in a similar manner, all of the results except 4 are significant at the 5% probability level. It may be inferred, therefore, that the selection of the odd sample of the triad was due more to the change in taste from sweetness than to any other factor.

SUMMARY AND CONCLUSIONS

An experienced taste panel was used to study the effect of MSG on the sweetness and taste of sucrose solutions. Each of several different sucrose levels was used as a base for admixture with increasing concentrations of MSG and the effect measured by an AA-B (triangle) test.

The MSG threshold for each level of sucrose was obtained by plotting concentrations of MSG against the percentage-above-chance scores on log-probability paper. At sucrose levels of 0.036, 0.072, 0.144, and 0.288 mole the corresponding MSG thresholds were 0.0018, 0.0025, 0.0035, and 0.0050 mole, respectively. When the concentration of sucrose was doubled, the factor of increase for MSG at threshold was 1.4.

Analysis of correct identifications with respect to effect on sweetness showed that MSG at sub-threshold levels did not enhance or depress the sweetness of sucrose.

There was evidence, however, that MSG-sucrose mixtures were identified because a change in taste not related to sweetness had been created by the MSG.

LITERATURE CITED

1. Harrison, S., and Elder, L. W. Some applications of statistics to laboratory taste testing. *Food Technol.*, **4**, 434 (1950).
2. Helm, E., and Trolle, B. Selection of a taste panel. *Wallerstein Labs. Commun.*, **9** (28), 181 (1946).
3. Lockhart, E. E. Binomial systems and organoleptic analysis. *Food Technol.*, **5**, 428 (1951).
4. Lockhart, E. E., and Gainer, J. M. Effect of monosodium glutamate on taste of pure sucrose and sodium chloride. *Food Research*, **15**, 459 (1950).
5. Snedecor, G. W. *Statistical Methods.* 4th Ed., 1946, Iowa State Coll. Press, Ames, Iowa.

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